Supplemental Material

Ferrocene Based Anilides: Synthesis, Structural Characterization and Inhibition of Butyrylcholinesterase

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Table of Content for supplemental material

In addition to this information, the X-ray data for compounds **M7** and **P9,** CIF files for these compounds have also been submitted to CCDC with numbers 1838788 and 1838789,respectively

checkCIF/PLATON report

You have not supplied any structure factors. As a result the full set of tests cannot be run.

THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE.

No syntax errors found. CIF dictionary Interpreting this report

Datablock: shelxl

```
Bond precision:  C-C = 0.0053 A          Wavelength=0.71073

Cell:           a=12.6321(19)    b=14.883(2)        c=10.2714(16)
                alpha=90         beta=105.383(4)    gamma=90
Temperature:    296 K

                Calculated                Reported
Volume          1861.9(5)                 1861.9(5)
Space group     P 21/c                    P21/c
Hall group      -P 2ybc                   -P2ybc
Moiety formula  C23 H18 Cl Fe N O         C23 H18 Cl Fe N O
Sum formula     C23 H18 Cl Fe N O         C23 H18 Cl Fe N O
Mr              415.68                    415.68
Dx,g cm-3       1.483                     1.483
Z               4                         4
Mu (mm-1)       0.966                     0.966
F000            856.0                     856.0
F000'           858.22
h,k,lmax        15,17,12                  15,17,12
Nref            3378                      3378
Tmin,Tmax       0.812,0.891               0.812,0.891
Tmin'           0.809

Correction method= # Reported T Limits: Tmin=0.812 Tmax=0.891
AbsCorr = MULTI-SCAN

Data completeness= 1.000          Theta(max)= 25.250

R(reflections)= 0.0416( 2363)     wR2(reflections)= 0.1168( 3327)

S = 1.022                 Npar= 244
```

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level C

```
ABSTY02_ALERT_1_C  An _exptl_absorpt_correction_type has been given without
           a literature citation. This should be contained in the
           _exptl_absorpt_process_details field.
           Absorption correction given as multi-scan
PLAT241_ALERT_2_C High   'MainMol' Ueq as Compared to Neighbors of         C1 Check
PLAT241_ALERT_2_C High   'MainMol' Ueq as Compared to Neighbors of         C5 Check
PLAT242_ALERT_2_C Low    'MainMol' Ueq as Compared to Neighbors of        Fe1 Check
PLAT242_ALERT_2_C Low    'MainMol' Ueq as Compared to Neighbors of        C18 Check
PLAT414_ALERT_2_C Short Intra D-H..H-X       H1     ..  H16     ..       1.96 Ang.
```

Alert level G

```
PLAT005_ALERT_5_G No Embedded Refinement Details found  in the CIF     Please Do !
PLAT007_ALERT_5_G Number of Unrefined Donor-H Atoms ..............          1 Report
PLAT066_ALERT_1_G Predicted and Reported Tmin&Tmax Range Identical          ? Check
PLAT093_ALERT_1_G No s.u.'s on H-positions, Refinement Reported as      mixed Check
```

```
   0 ALERT level A = Most likely a serious problem - resolve or explain
   0 ALERT level B = A potentially serious problem, consider carefully
   6 ALERT level C = Check. Ensure it is not caused by an omission or oversight
   4 ALERT level G = General information/check it is not something unexpected

   3 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
   5 ALERT type 2 Indicator that the structure model may be wrong or deficient
   0 ALERT type 3 Indicator that the structure quality may be low
   0 ALERT type 4 Improvement, methodology, query or suggestion
   2 ALERT type 5 Informative message, check
```

It is advisable to attempt to resolve as many as possible of the alerts in all categories. Often the minor alerts point to easily fixed oversights, errors and omissions in your CIF or refinement strategy, so attention to these fine details can be worthwhile. In order to resolve some of the more serious problems it may be necessary to carry out additional measurements or structure refinements. However, the purpose of your study may justify the reported deviations and the more serious of these should normally be commented upon in the discussion or experimental section of a paper or in the "special_details" fields of the CIF. checkCIF was carefully designed to identify outliers and unusual parameters, but every test has its limitations and alerts that are not important in a particular case may appear. Conversely, the absence of alerts does not guarantee there are no aspects of the results needing attention. It is up to the individual to critically assess their own results and, if necessary, seek expert advice.

Publication of your CIF in IUCr journals

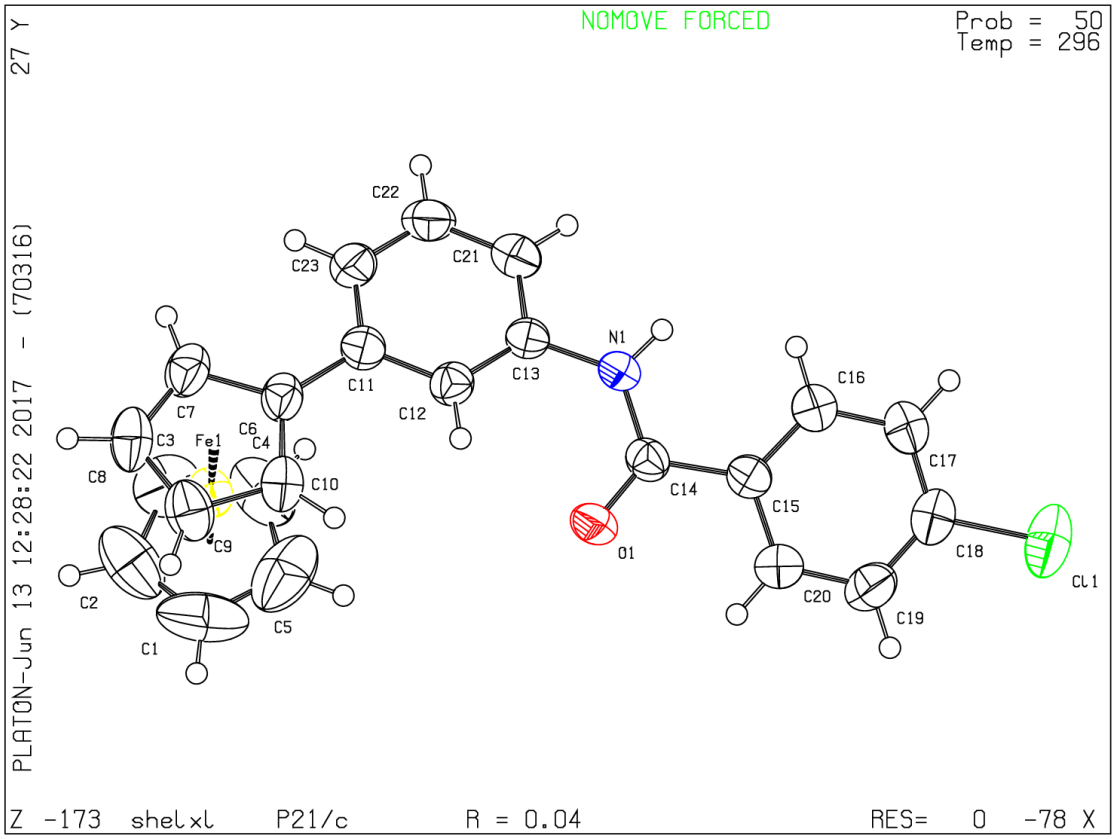
A basic structural check has been run on your CIF. These basic checks will be run on all CIFs submitted for publication in IUCr journals (*Acta Crystallographica*, *Journal of Applied Crystallography*, *Journal of Synchrotron Radiation*); however, if you intend to submit to *Acta Crystallographica Section C* or *E* or *IUCrData*, you should make sure that full publication checks are run on the final version of your CIF prior to submission.

Publication of your CIF in other journals

Please refer to the *Notes for Authors* of the relevant journal for any special instructions relating to CIF submission.

PLATON version of 27/03/2017; check.def file version of 24/03/2017

Datablock shelxl - ellipsoid plot



checkCIF/PLATON report

You have not supplied any structure factors. As a result the full set of tests cannot be run.

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No syntax errors found. CIF dictionary Interpreting this report

Datablock: shelxl

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Bond precision:  C-C = 0.0044 A          Wavelength=0.71073

Cell:            a=5.8071(3)    b=23.9440(12)       c=13.5031(9)
                 alpha=90       beta=101.921(3)     gamma=90
Temperature:     296 K
```

	Calculated	Reported
Volume	1837.05(18)	1837.05(18)
Space group	P 21/n	P21/n
Hall group	-P 2yn	-P2yn
Moiety formula	C24 H21 Fe N O	C24 H21 Fe N O
Sum formula	C24 H21 Fe N O	C24 H21 Fe N O
Mr	395.27	395.27
Dx,g cm-3	1.429	1.429
Z	4	4
Mu (mm-1)	0.834	0.834
F000	824.0	824.0
F000’	825.63	
h,k,lmax	6,28,16	6,28,16
Nref	3307	3307
Tmin,Tmax	0.835,0.905	0.835,0.905
Tmin’	0.819	

```
Correction method= # Reported T Limits: Tmin=0.835 Tmax=0.905
AbsCorr = MULTI-SCAN

Data completeness= 1.000          Theta(max)= 25.250

R(reflections)= 0.0386( 2423)     wR2(reflections)= 0.0914( 3305)

S = 1.016                 Npar= 245
```

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level C

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ABSTY02_ALERT_1_C  An _exptl_absorpt_correction_type has been given without
           a literature citation. This should be contained in the
           _exptl_absorpt_process_details field.
           Absorption correction given as multi-scan
PLAT420_ALERT_2_C D-H Without Acceptor       N1     --  H1     ...     Please Check
```

Alert level G

```
PLAT005_ALERT_5_G No Embedded Refinement Details found  in the CIF     Please Do !
PLAT007_ALERT_5_G Number of Unrefined Donor-H Atoms ..............          1 Report
PLAT066_ALERT_1_G Predicted and Reported Tmin&Tmax Range Identical          ? Check
PLAT093_ALERT_1_G No s.u.'s on H-positions, Refinement Reported as       mixed Check
```

```
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   3 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
   1 ALERT type 2 Indicator that the structure model may be wrong or deficient
   0 ALERT type 3 Indicator that the structure quality may be low
   0 ALERT type 4 Improvement, methodology, query or suggestion
   2 ALERT type 5 Informative message, check
```

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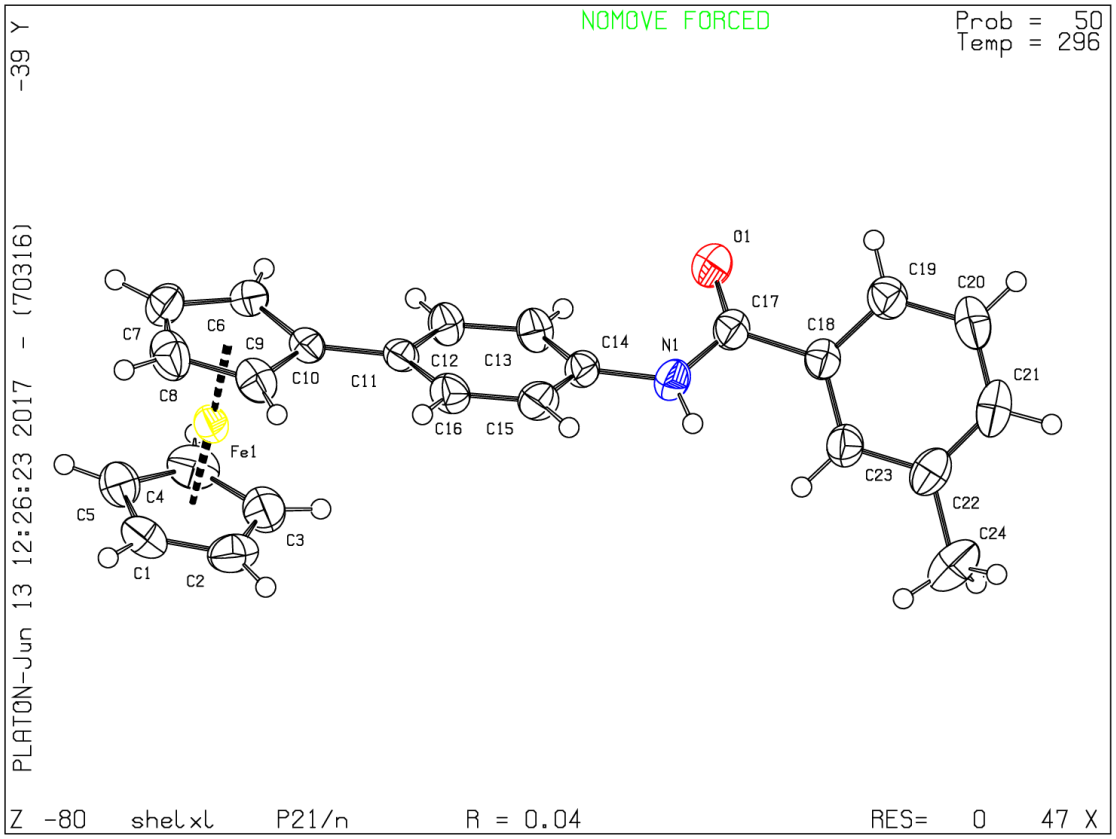
PLATON version of 27/03/2017; check.def file version of 24/03/2017

Datablock shelxl - ellipsoid plot



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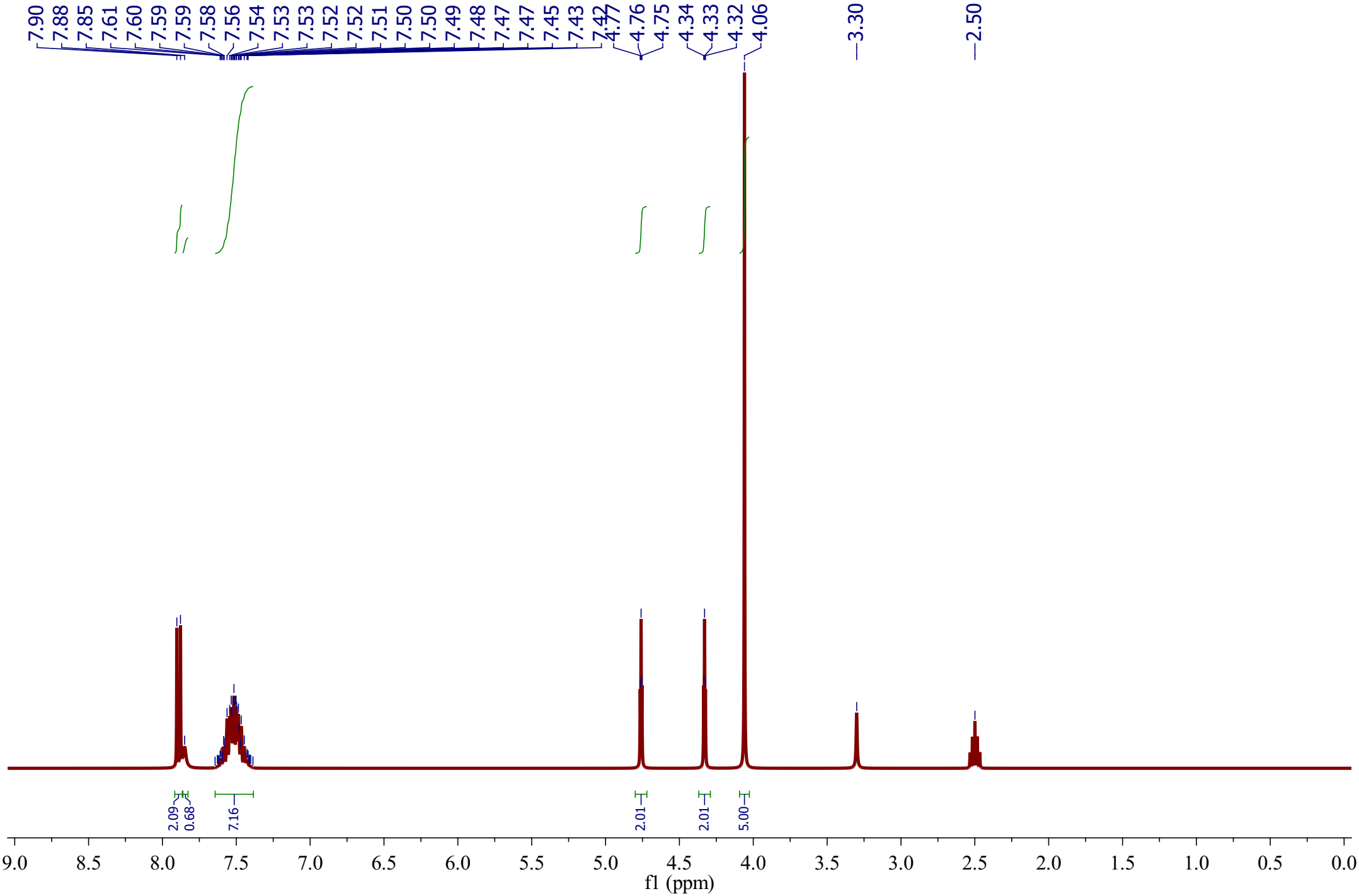
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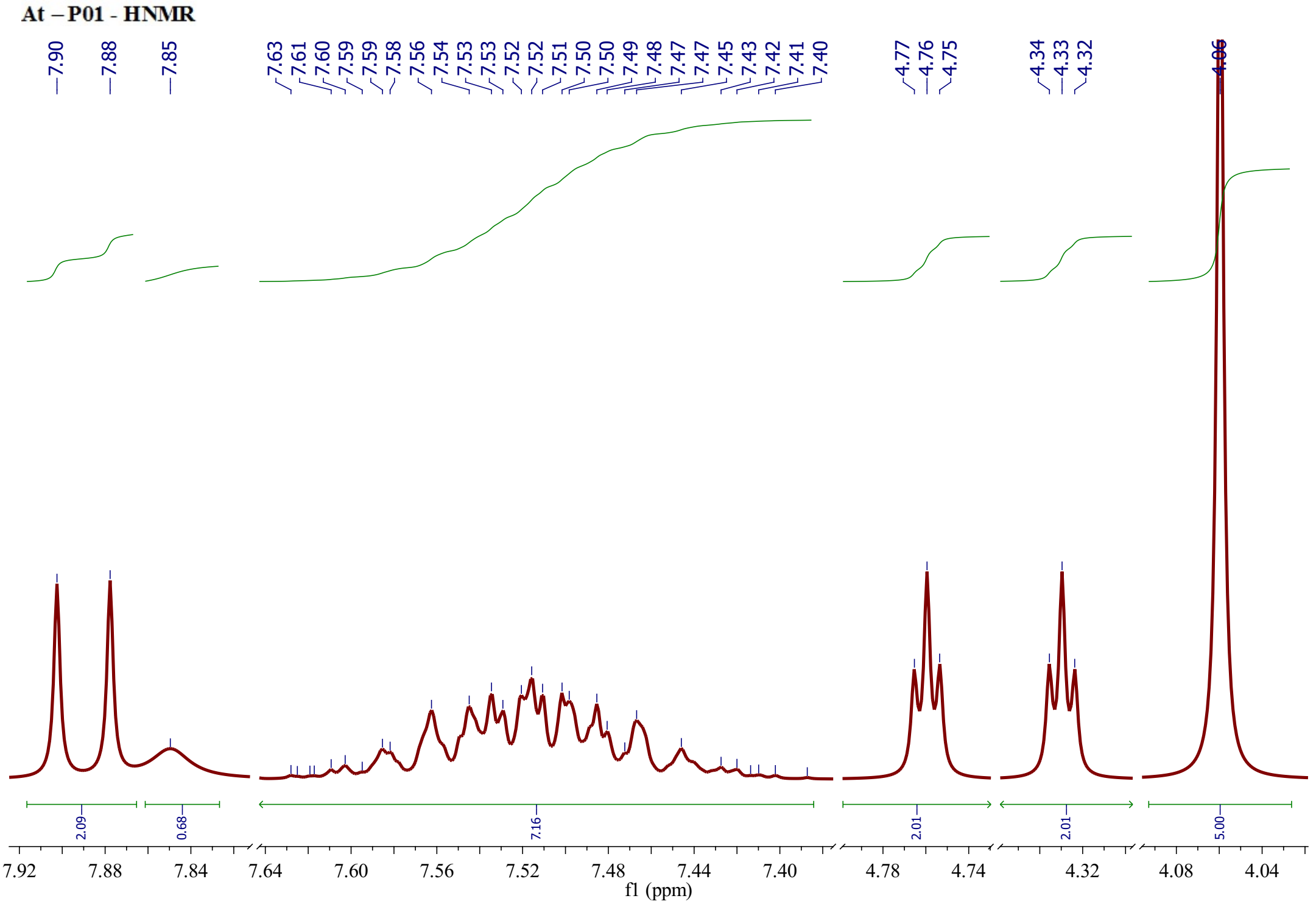
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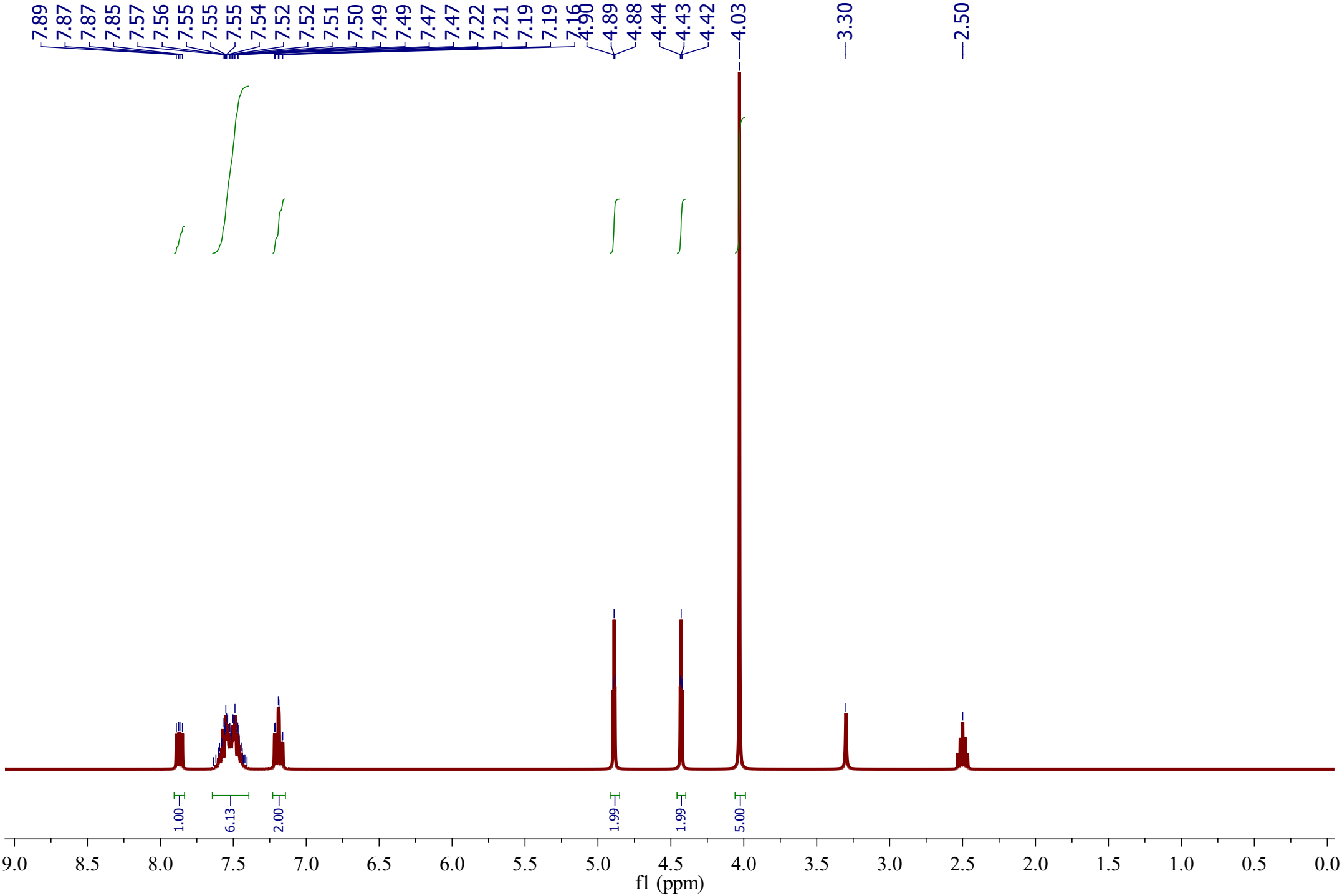
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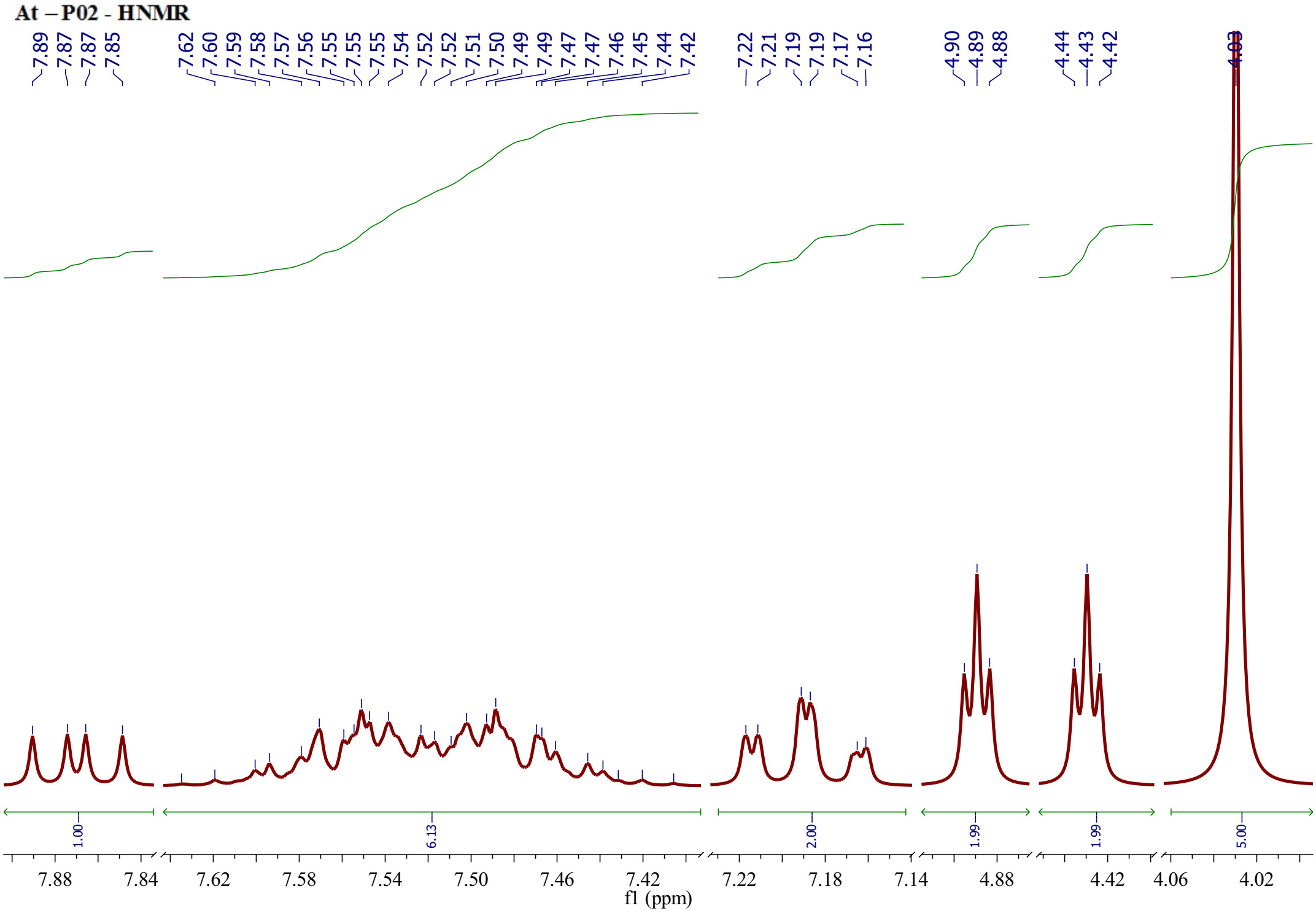
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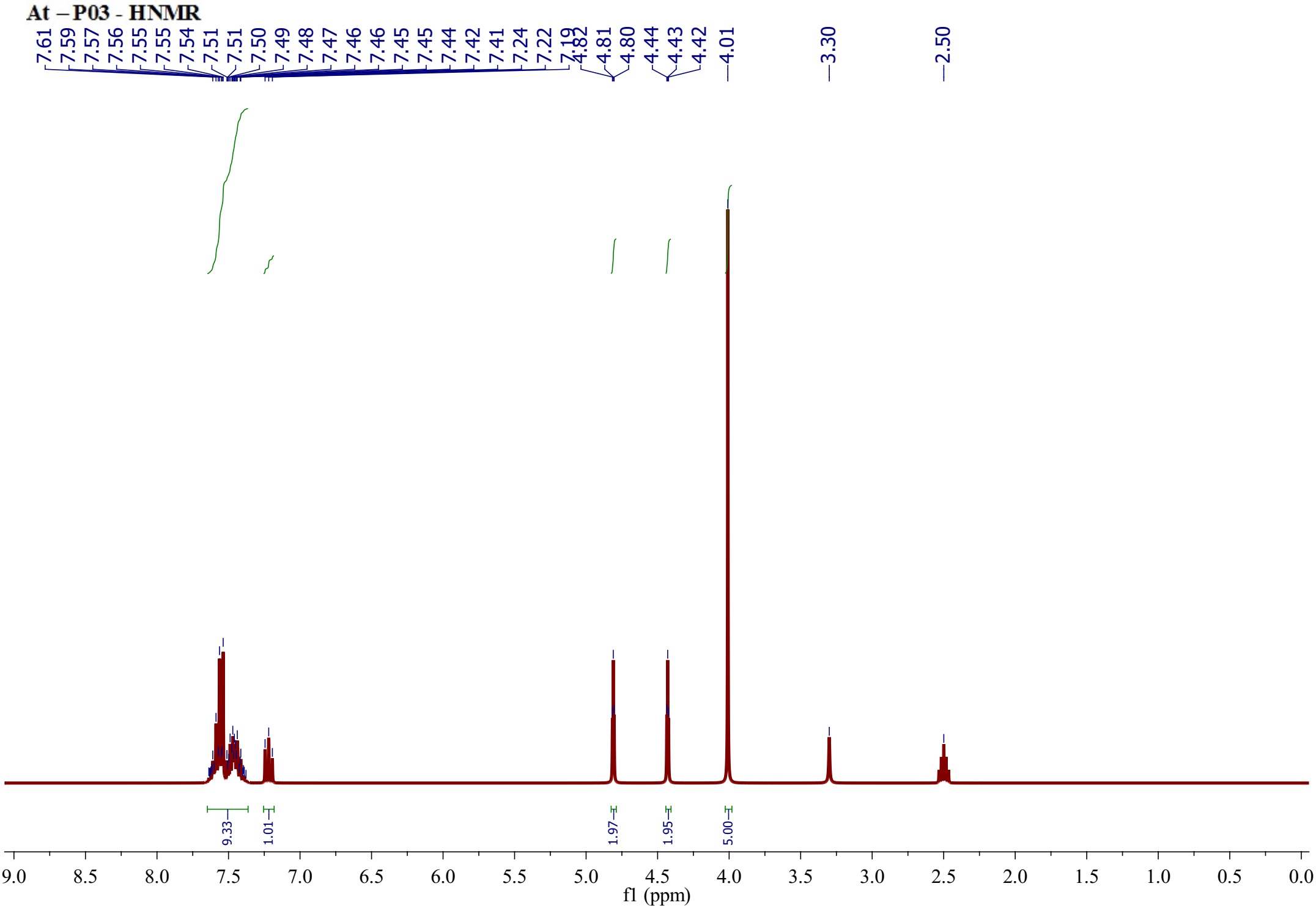
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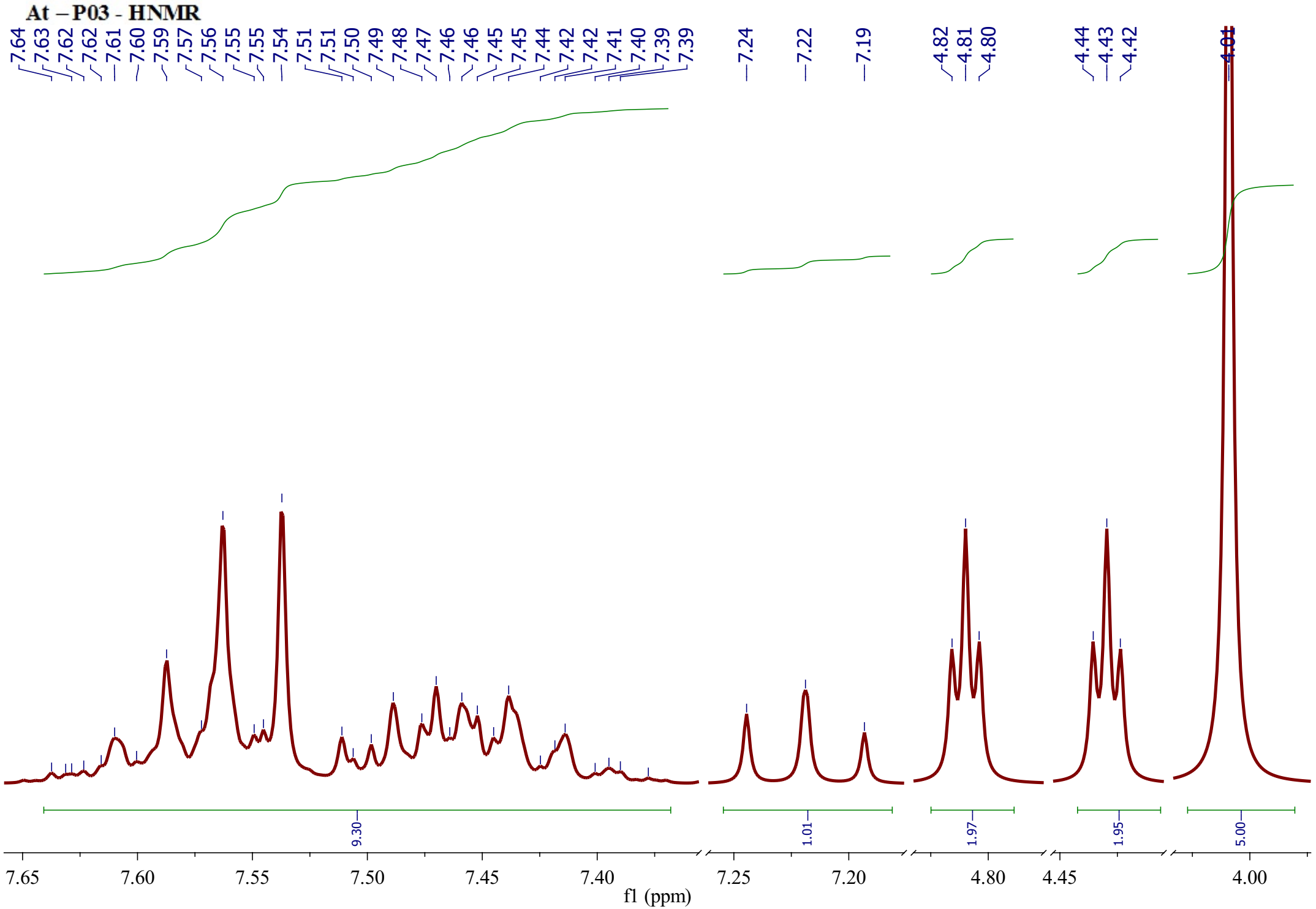
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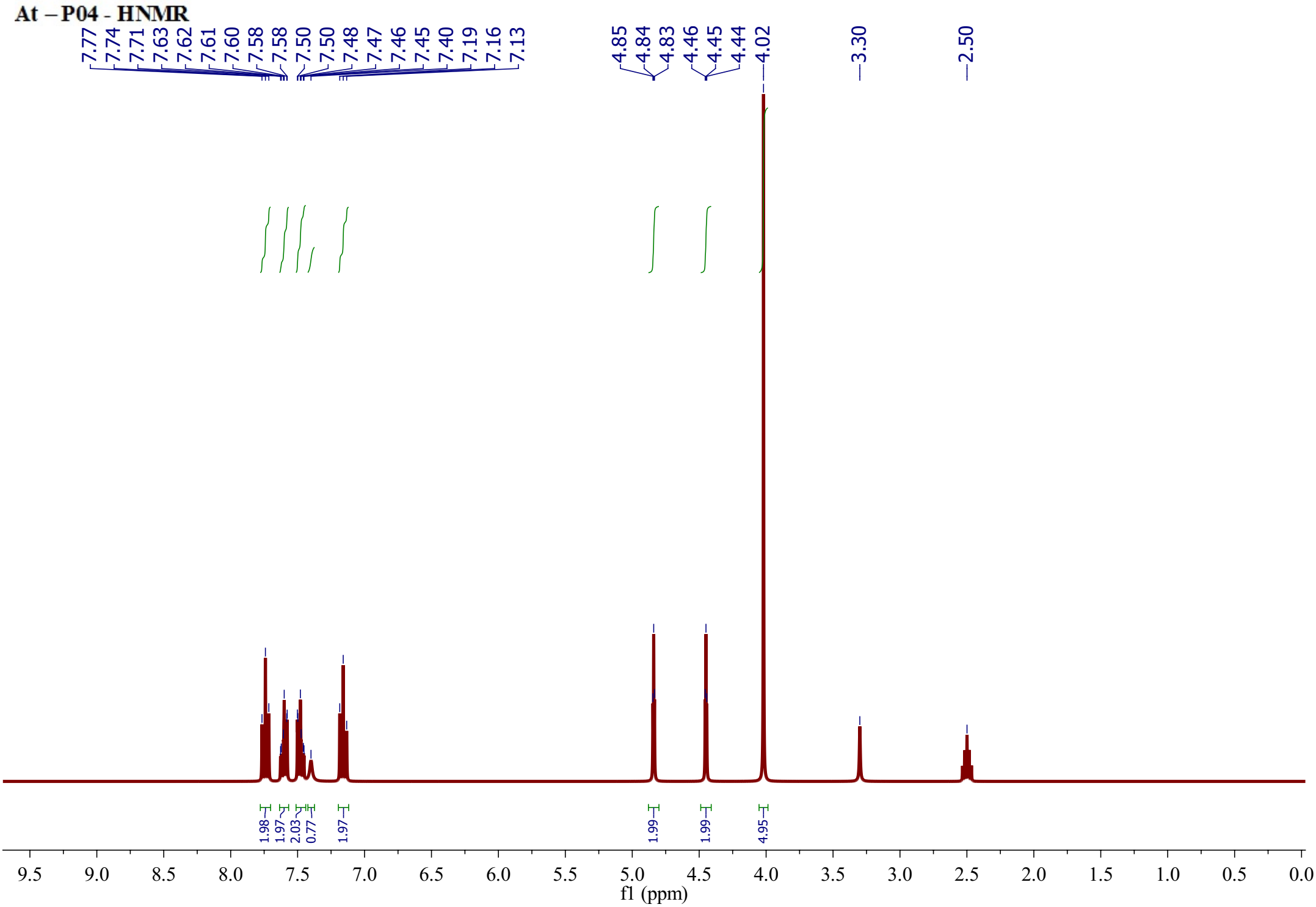
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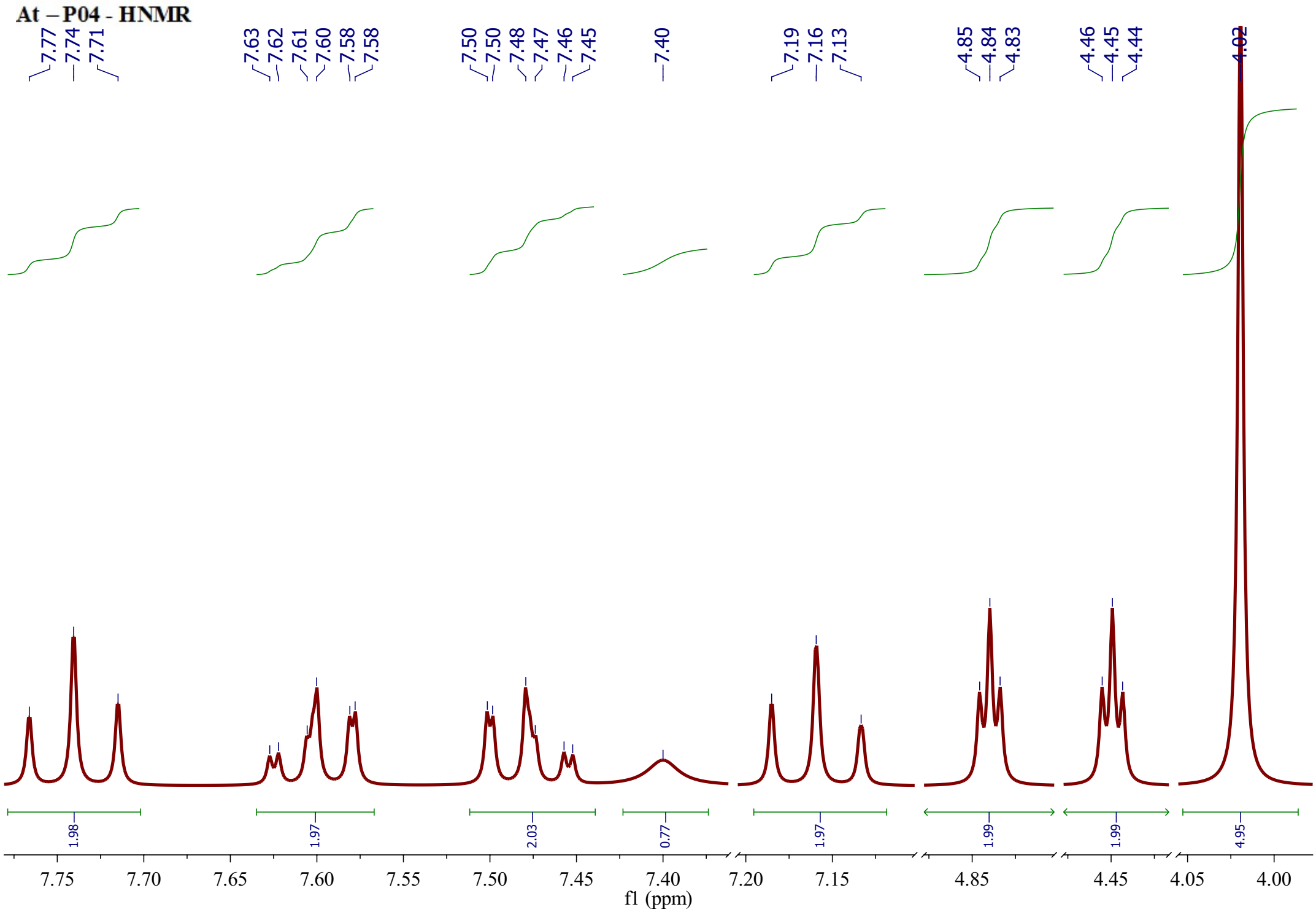
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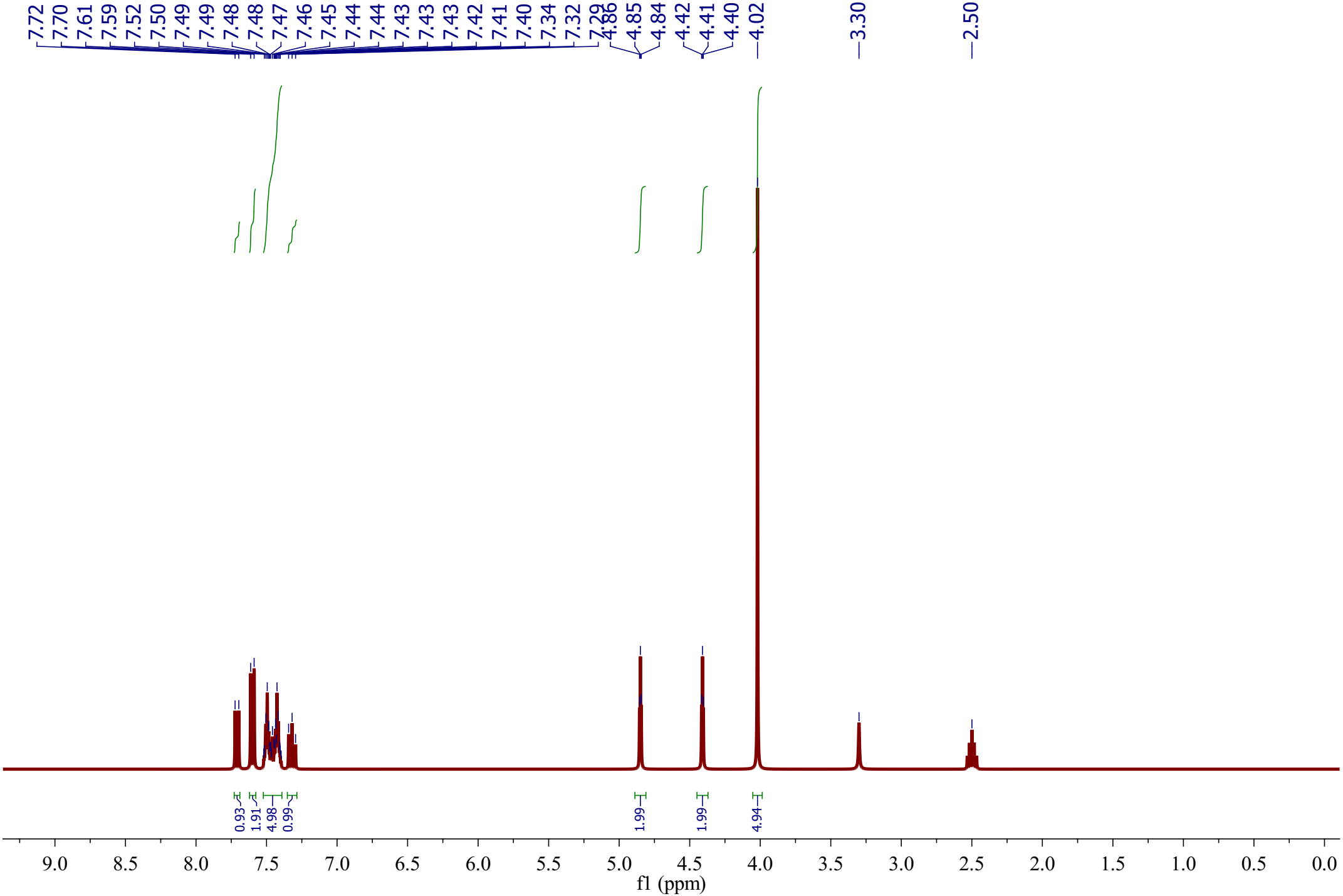
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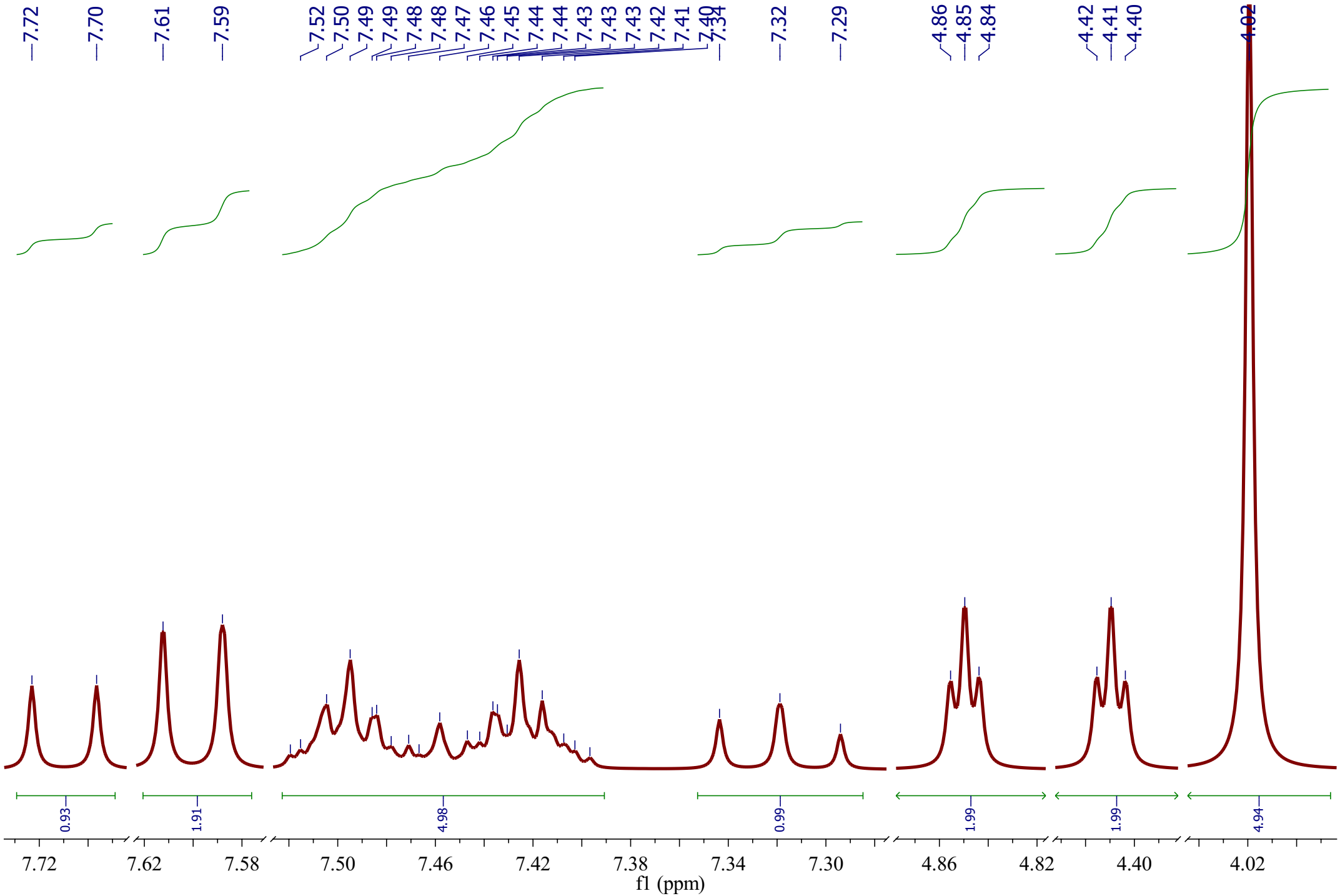
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4.94
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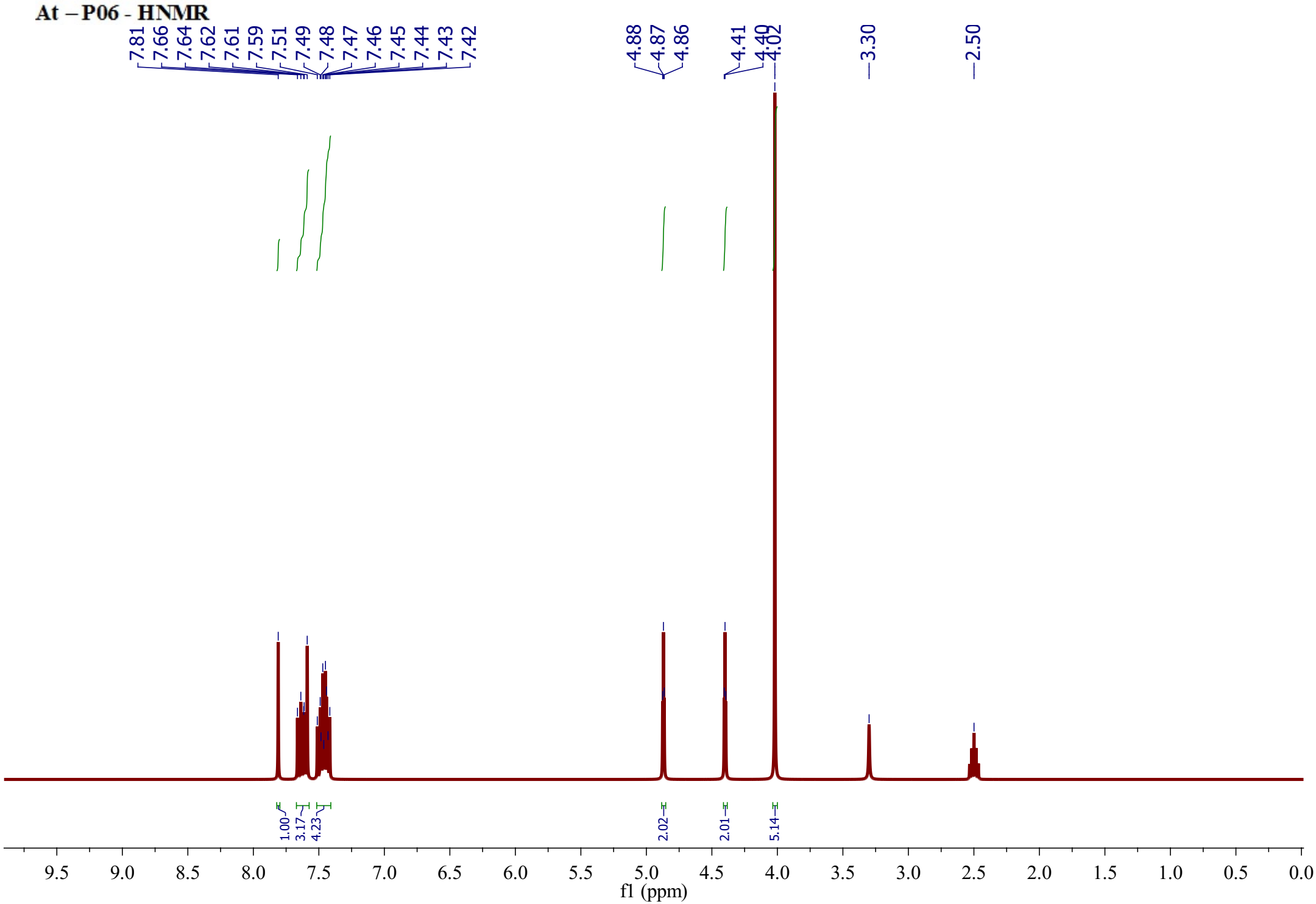
At – P06 - HNMR
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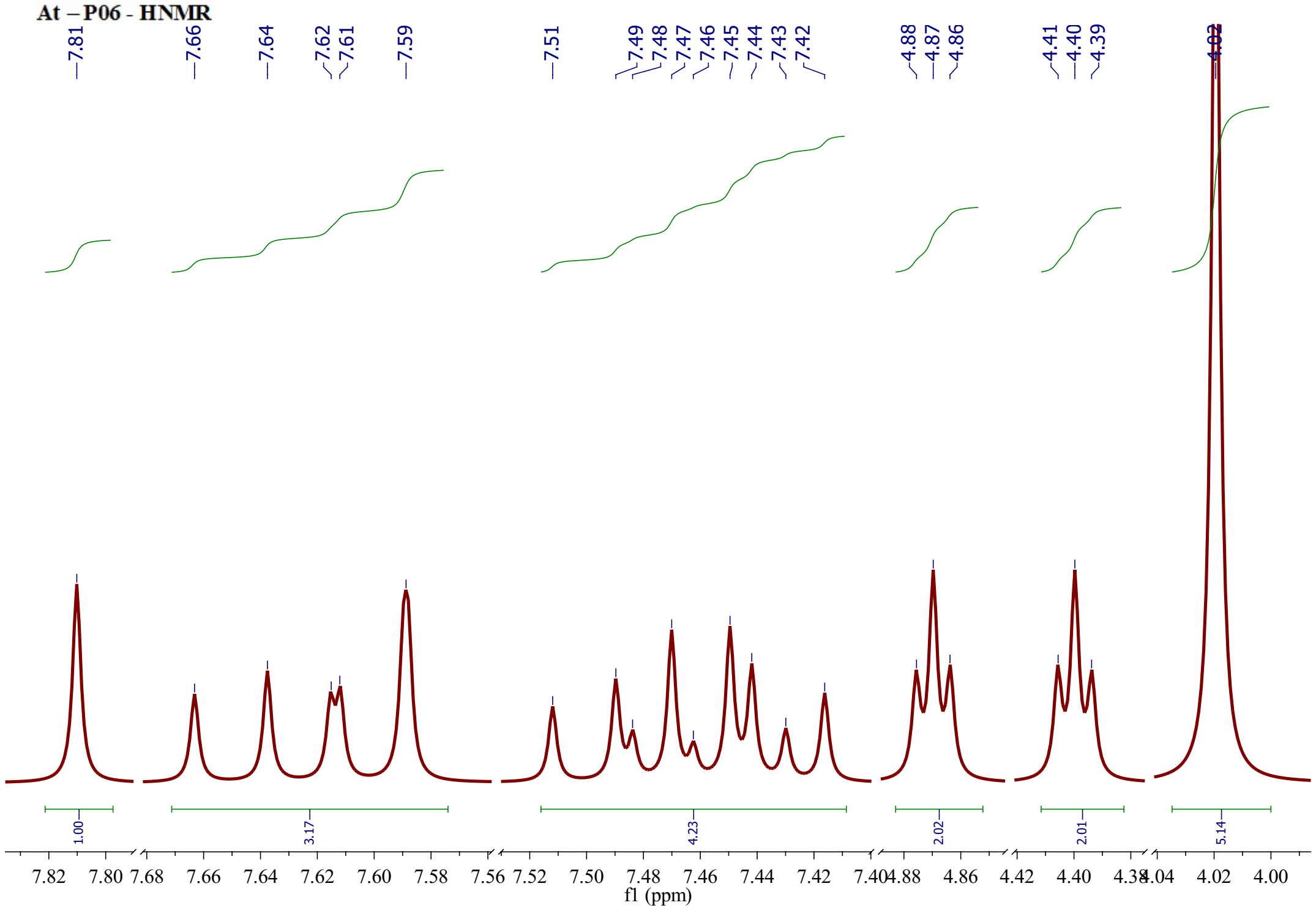
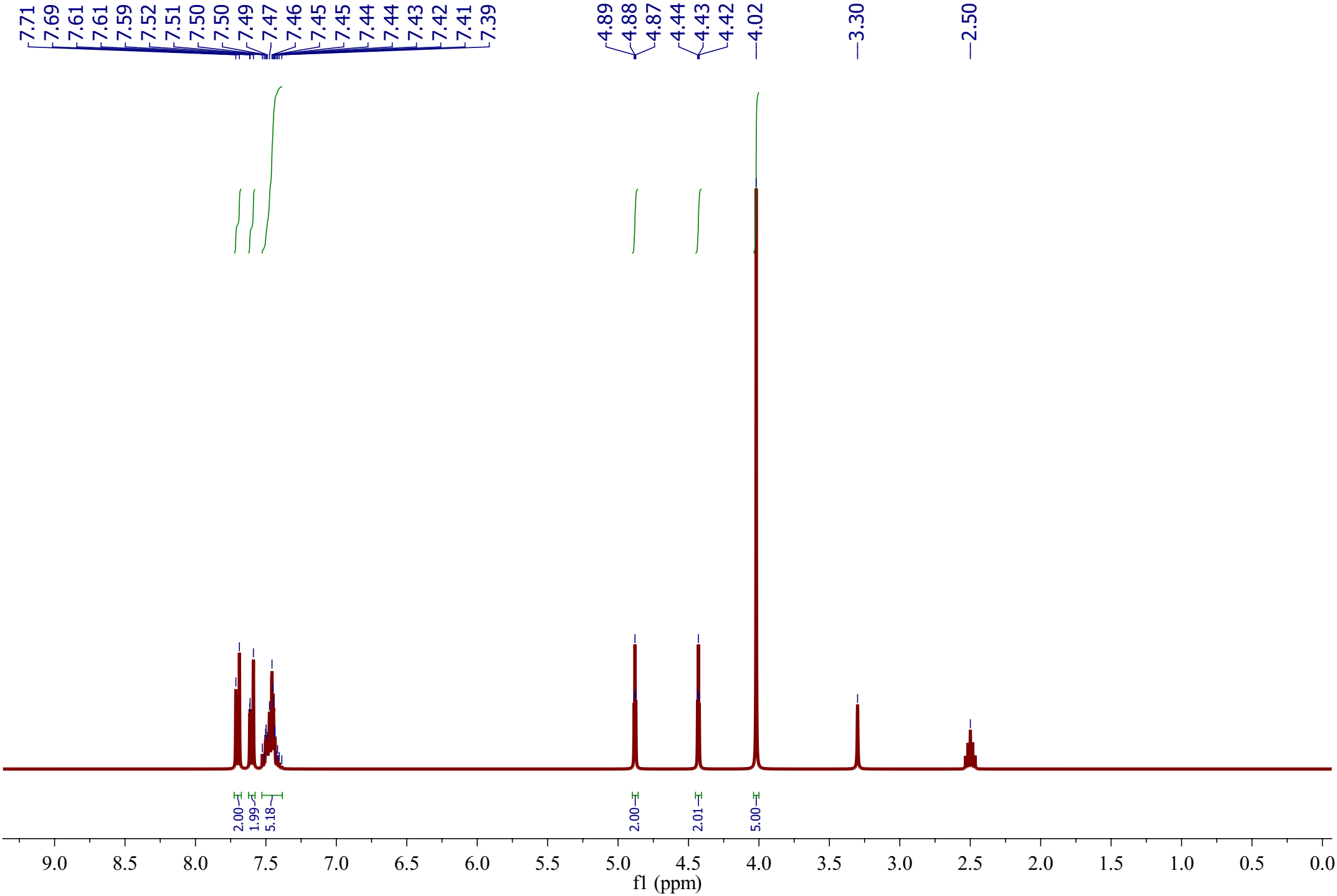
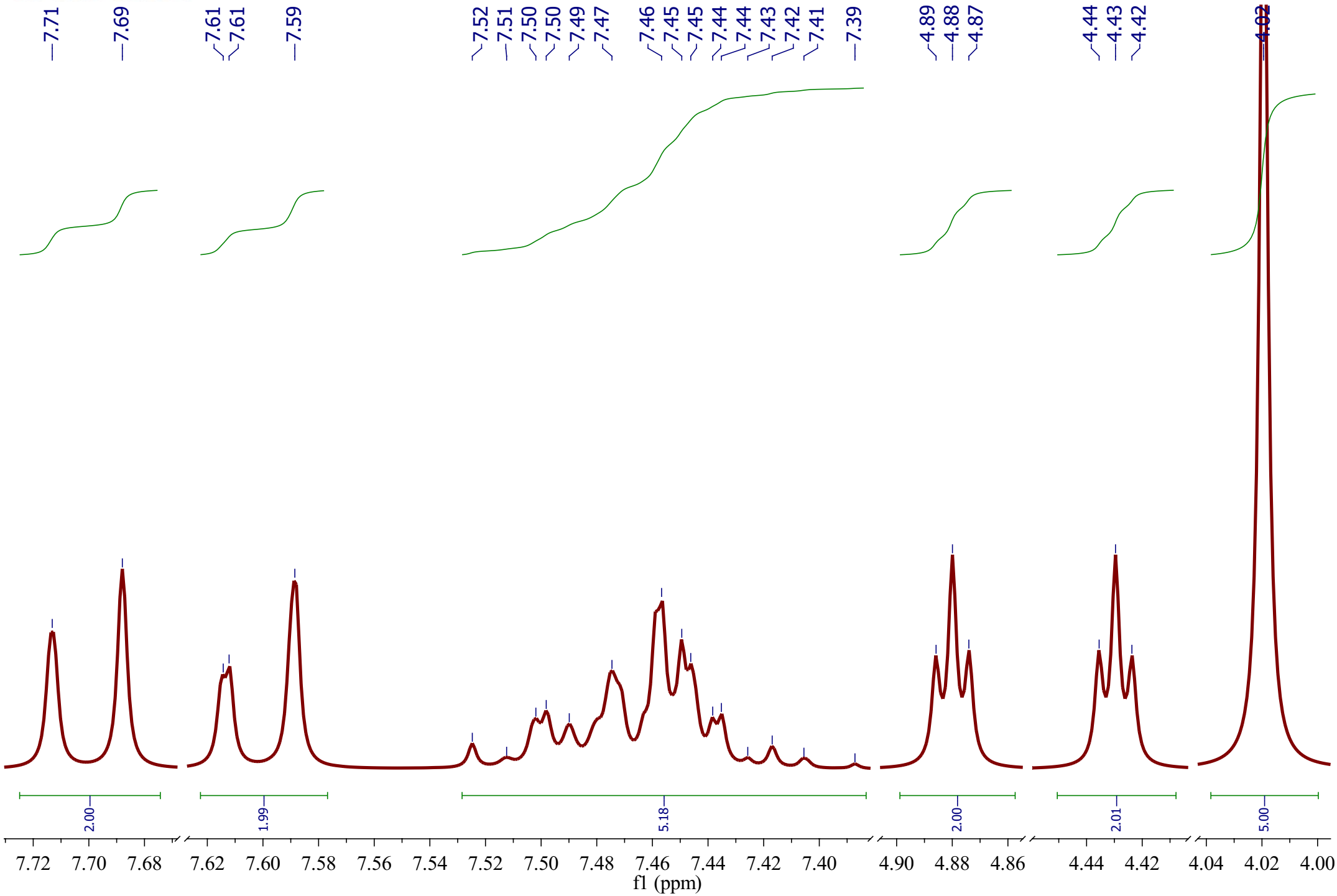
7.49
7.48
7.47
7.46
7.45
7.44
7.43
7.42
4.88
4.87
4.86
4.41
4.40
4.02
3.30
2.50
1.00
3.17
4.23
2.02
2.01
5.14
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

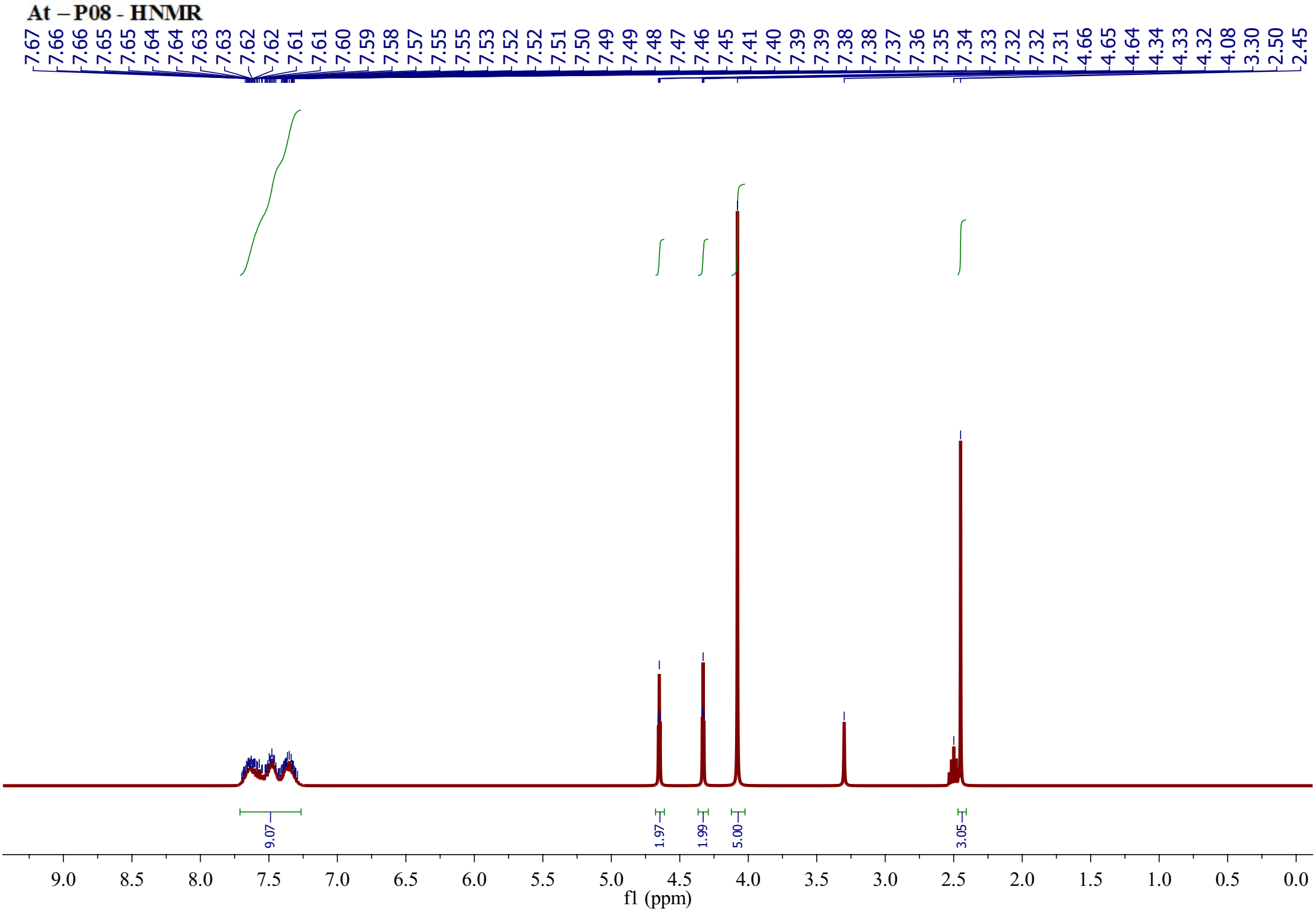

At – P06 - HNMR
7.81
7.66
7.64
7.62
7.61
7.59
7.51
7.49
7.48
7.47
7.46
7.45
7.44
7.43
7.42
4.88
4.87
4.86
4.41
4.40
4.39
4.02
1.00
3.17
4.23
2.02
2.01
5.14
7.82 7.80 7.68 7.66 7.64 7.62 7.60 7.58 7.56 7.52 7.50 7.48 7.46 7.44 7.42 7.40 4.88 4.86 4.42 4.40 4.38 4.04 4.02 4.00
f1 (ppm)

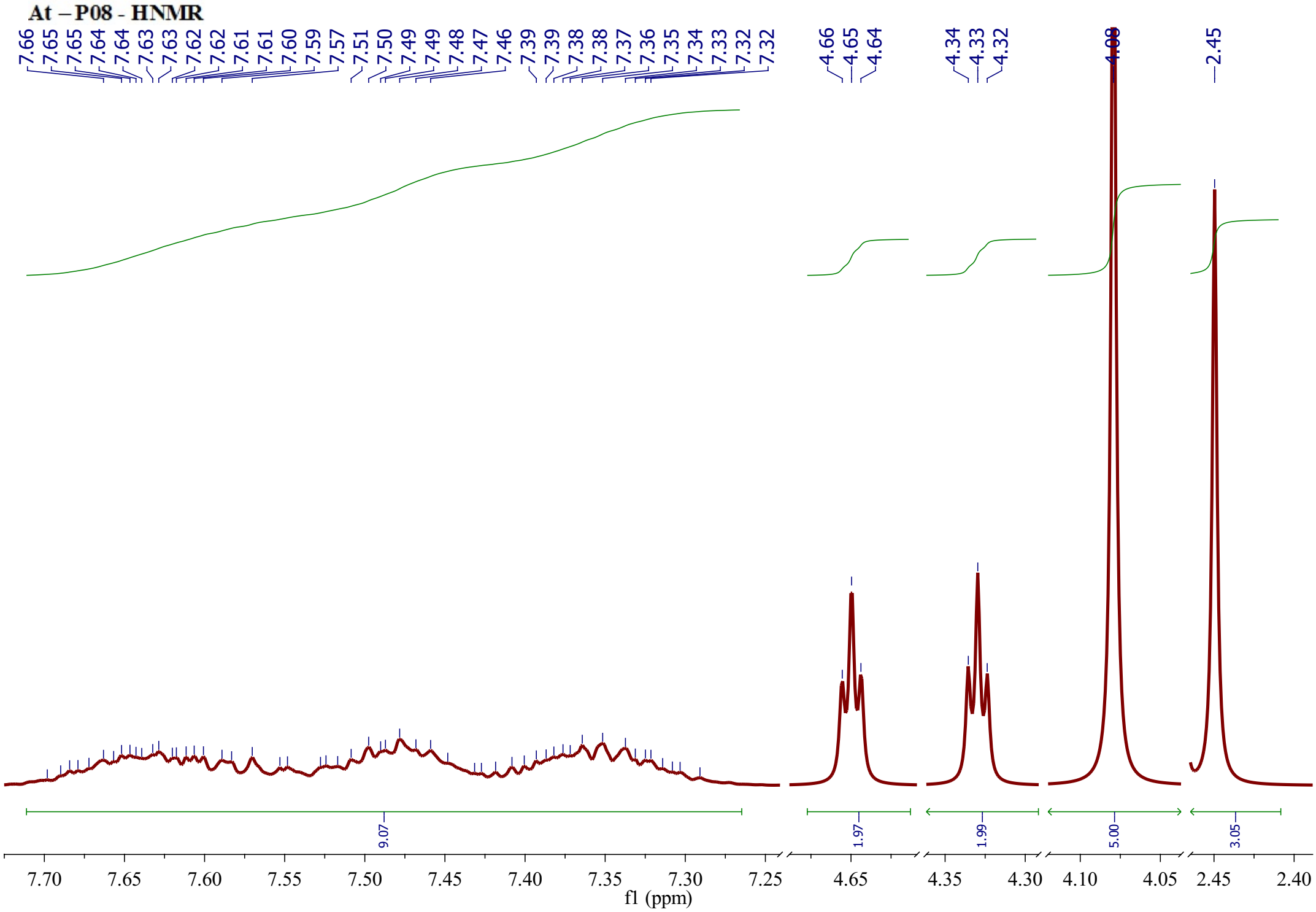
At – P07 - HNMR

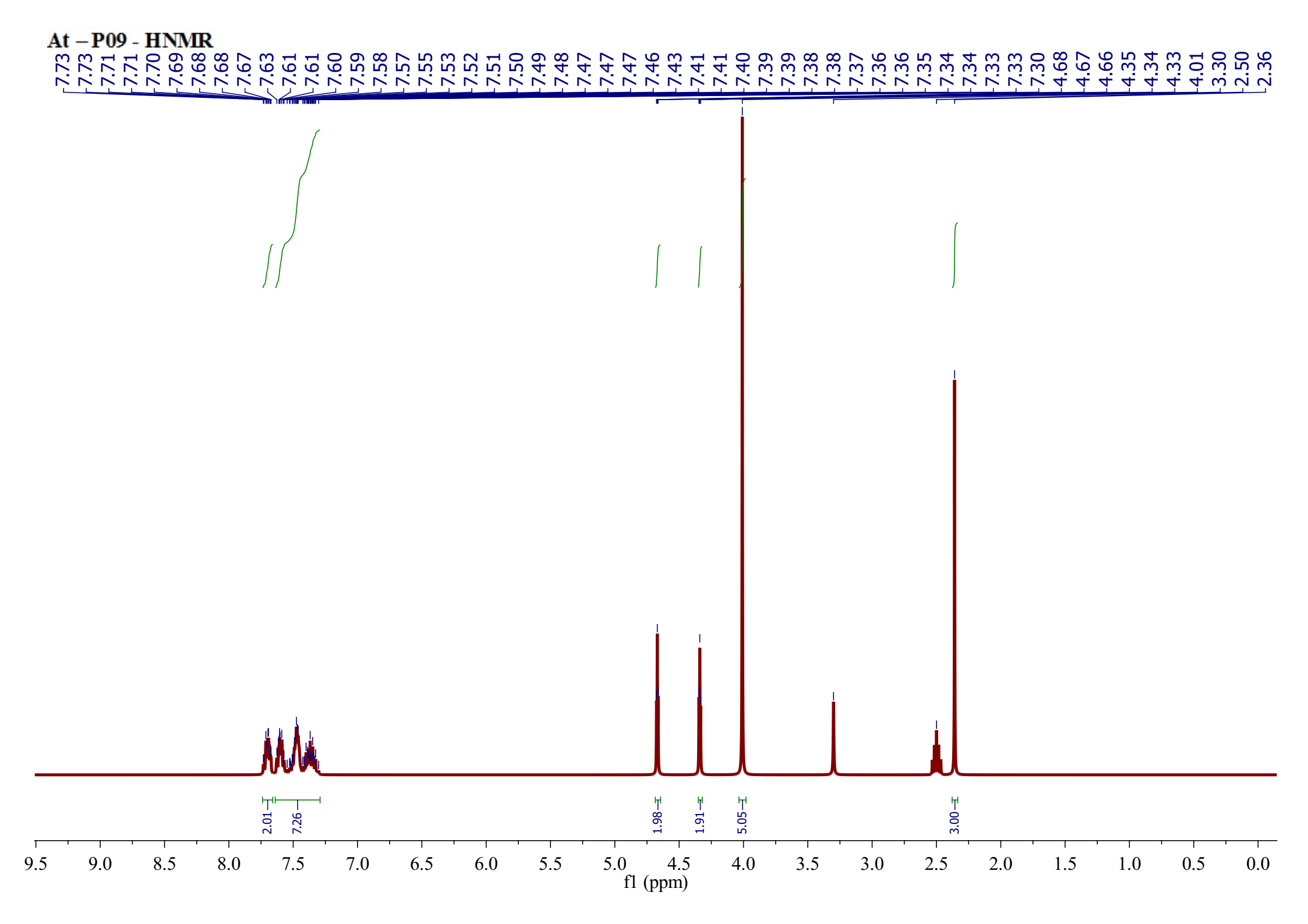

7.71
7.69
7.61
7.61
7.59
7.52
7.51
7.50
7.50
7.49
7.47
7.46
7.45
7.45
7.44
7.44
7.43
7.42
7.41
7.39
4.89
4.88
4.87
4.44
4.43
4.42
4.02
3.30
2.50
2.00
1.99
5.18
2.00
2.01
5.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

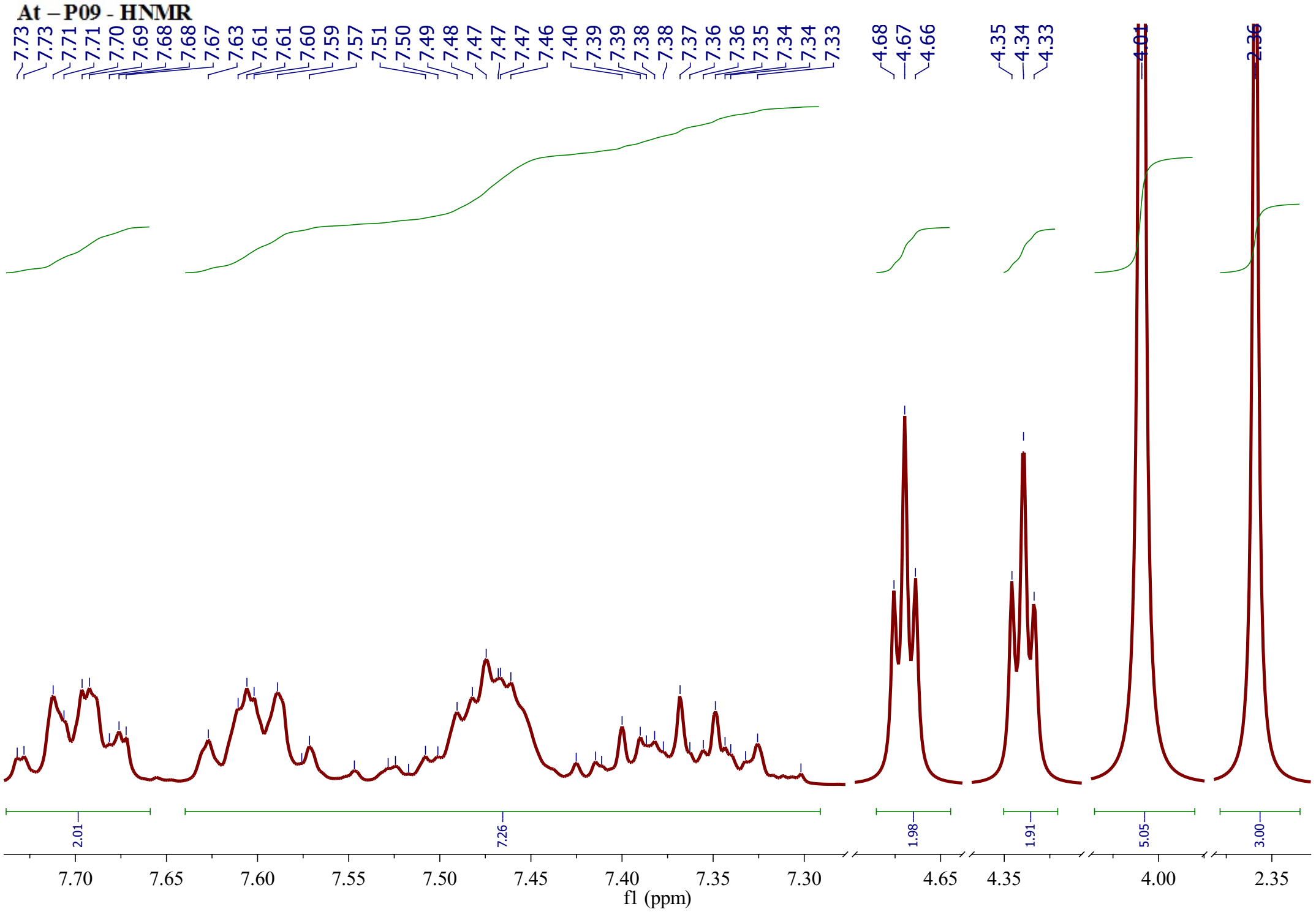
At – P07 - HNMR


7.71
7.69
7.61
7.61
7.59
7.52
7.51
7.50
7.50
7.49
7.47
7.46
7.45
7.45
7.44
7.44
7.43
7.42
7.41
7.39
4.89
4.88
4.87
4.44
4.43
4.42
4.02
2.00
1.99
5.18
2.00
2.01
5.00
7.72 7.70 7.68 7.62 7.60 7.58 7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 4.90 4.88 4.86 4.44 4.42 4.04 4.02 4.00
f1 (ppm)

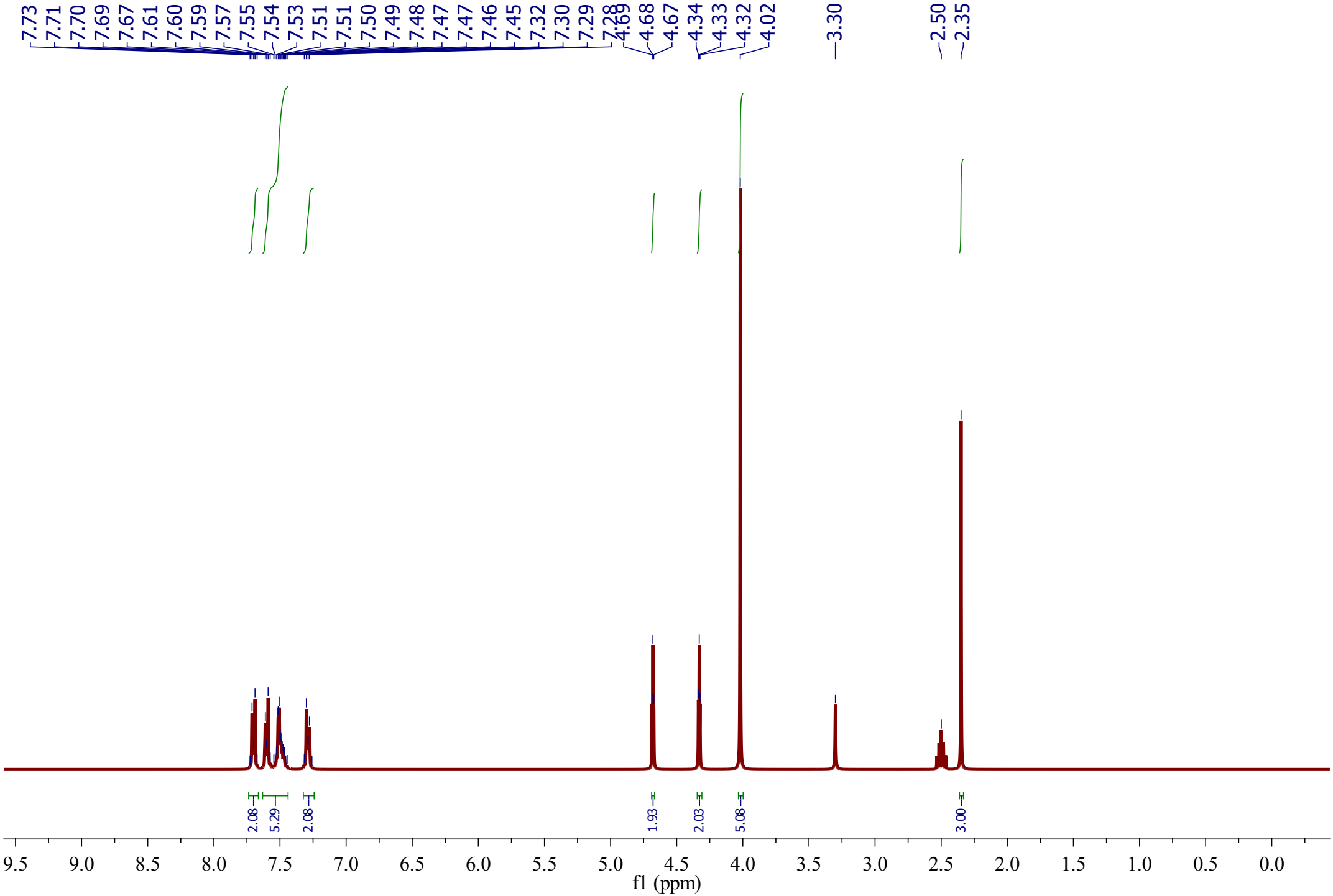

At – P08 - HNMR
7.67 7.66 7.66 7.65 7.65 7.64 7.64 7.63 7.63 7.62 7.62 7.61 7.61 7.60 7.59 7.58 7.57 7.55 7.55 7.53 7.52 7.52 7.51 7.50 7.49 7.49 7.48 7.47 7.46 7.45 7.41 7.40 7.39 7.39 7.38 7.38 7.37 7.36 7.35 7.34 7.33 7.32 7.32 7.31 4.66 4.65 4.64 4.34 4.33 4.32 4.08 3.30 2.50 2.45
9.07
1.97
1.99
5.00
3.05
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


At – P08 - HNMR
7.66
7.65
7.65
7.64
7.64
7.63
7.63
7.62
7.62
7.61
7.61
7.60
7.59
7.57
7.51
7.50
7.49
7.49
7.48
7.47
7.46
7.39
7.39
7.38
7.38
7.37
7.36
7.35
7.34
7.33
7.32
7.32
4.66
4.65
4.64
4.34
4.33
4.32
4.08
2.45
9.07
1.97
1.99
5.00
3.05
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.25
4.65
4.35
4.30
4.10
4.05
2.45
2.40
f1 (ppm)

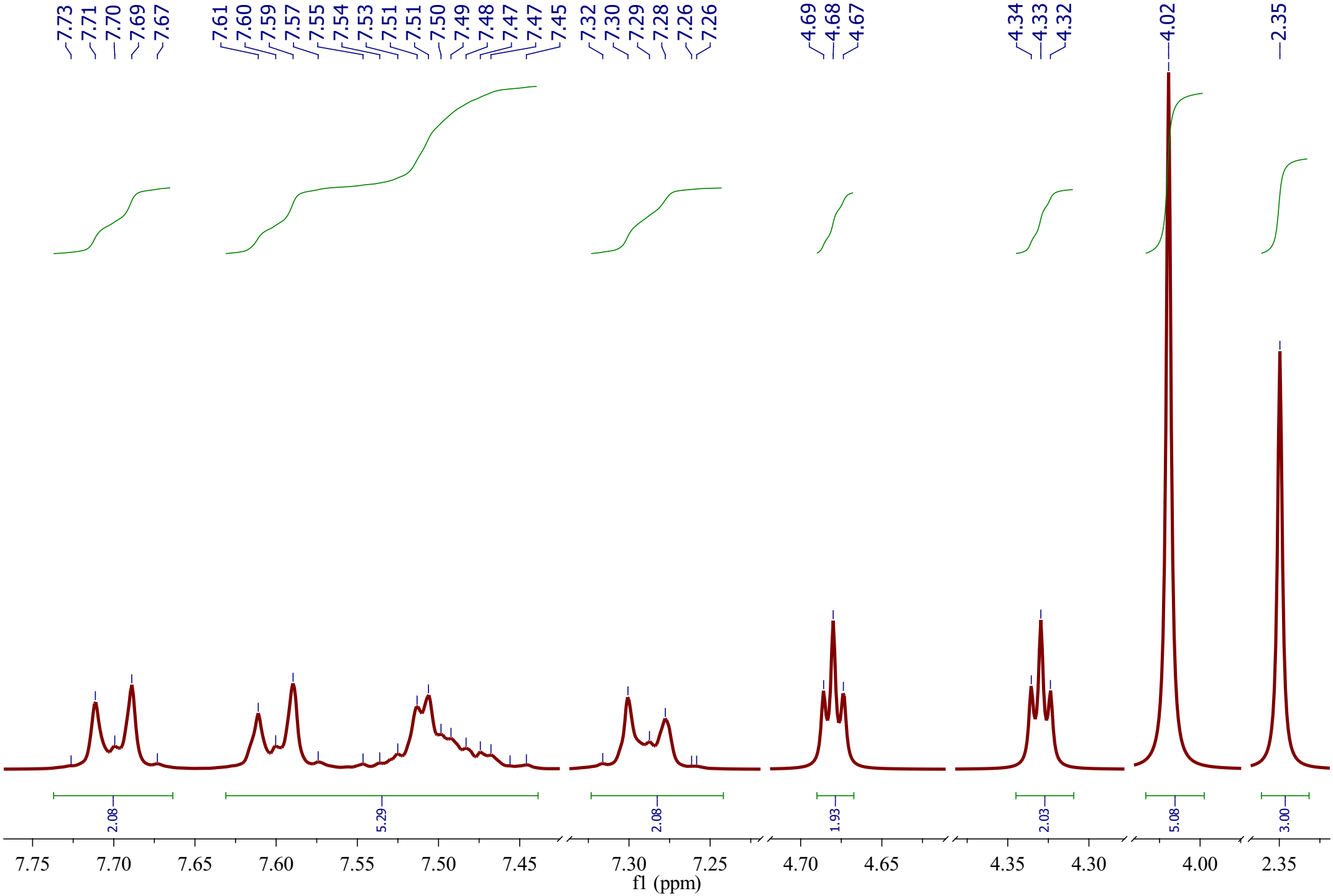

At – P09 - HNMR
7.73 7.73 7.71 7.71 7.70 7.69 7.68 7.68 7.67 7.63 7.61 7.61 7.60 7.59 7.58 7.57 7.55 7.53 7.52 7.51 7.50 7.49 7.48 7.47 7.47 7.47 7.46 7.43 7.41 7.41 7.40 7.39 7.39 7.38 7.38 7.37 7.36 7.36 7.35 7.34 7.34 7.33 7.33 7.30 4.68 4.67 4.66 4.35 4.34 4.33 4.01 3.30 2.50 2.36
2.01
7.26
1.98
1.91
5.05
3.00
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


At – P09 - HNMR
7.73
7.73
7.71
7.71
7.70
7.69
7.68
7.68
7.67
7.63
7.61
7.61
7.60
7.59
7.57
7.51
7.50
7.49
7.48
7.47
7.47
7.47
7.46
7.40
7.39
7.39
7.38
7.38
7.37
7.36
7.36
7.35
7.34
7.34
7.33
4.68
4.67
4.66
4.35
4.34
4.33
4.01
2.36
2.01
7.26
1.98
1.91
5.05
3.00
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
4.65
4.35
4.00
2.35
f1 (ppm)

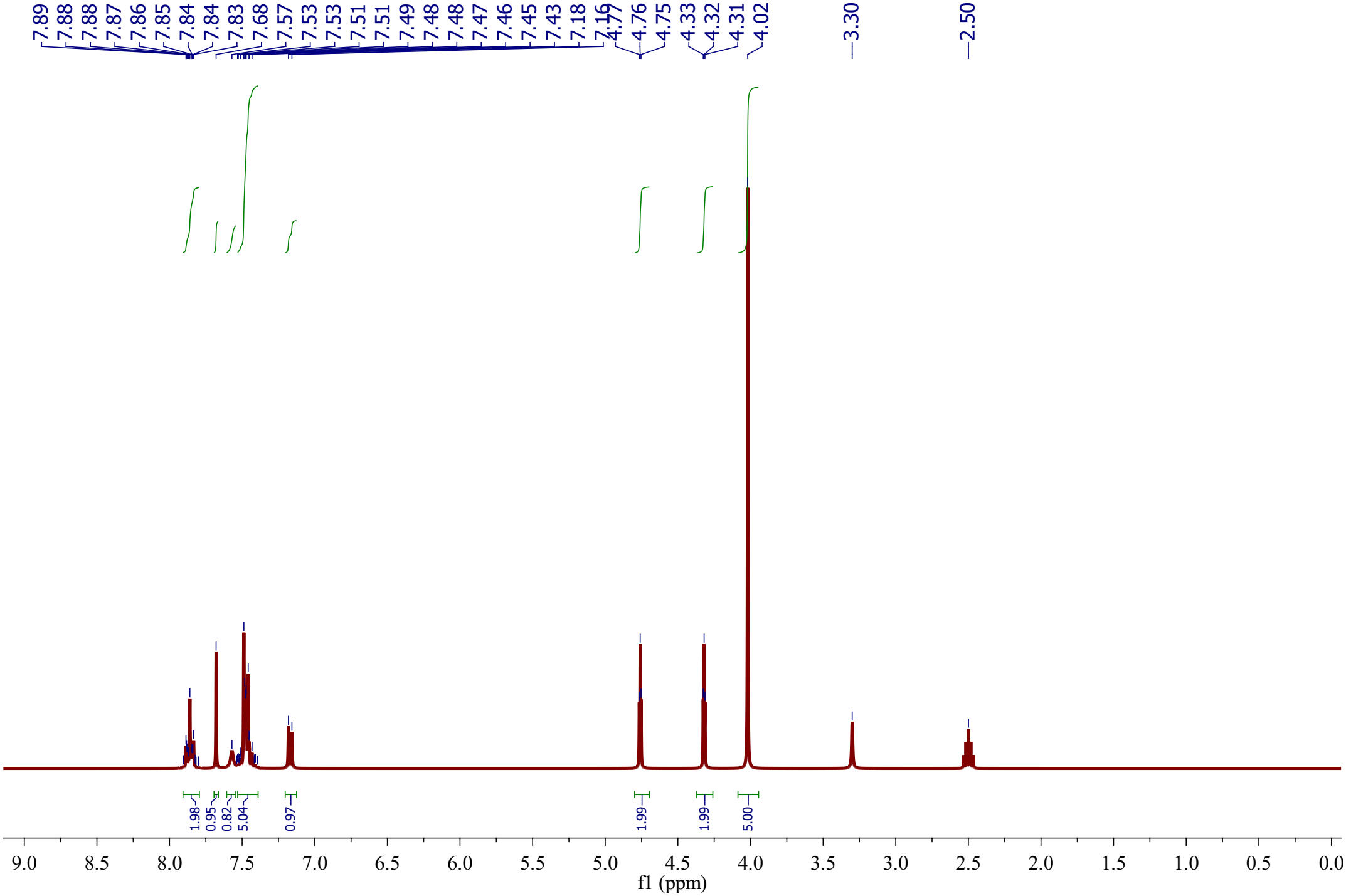
At – P10 - HNMR

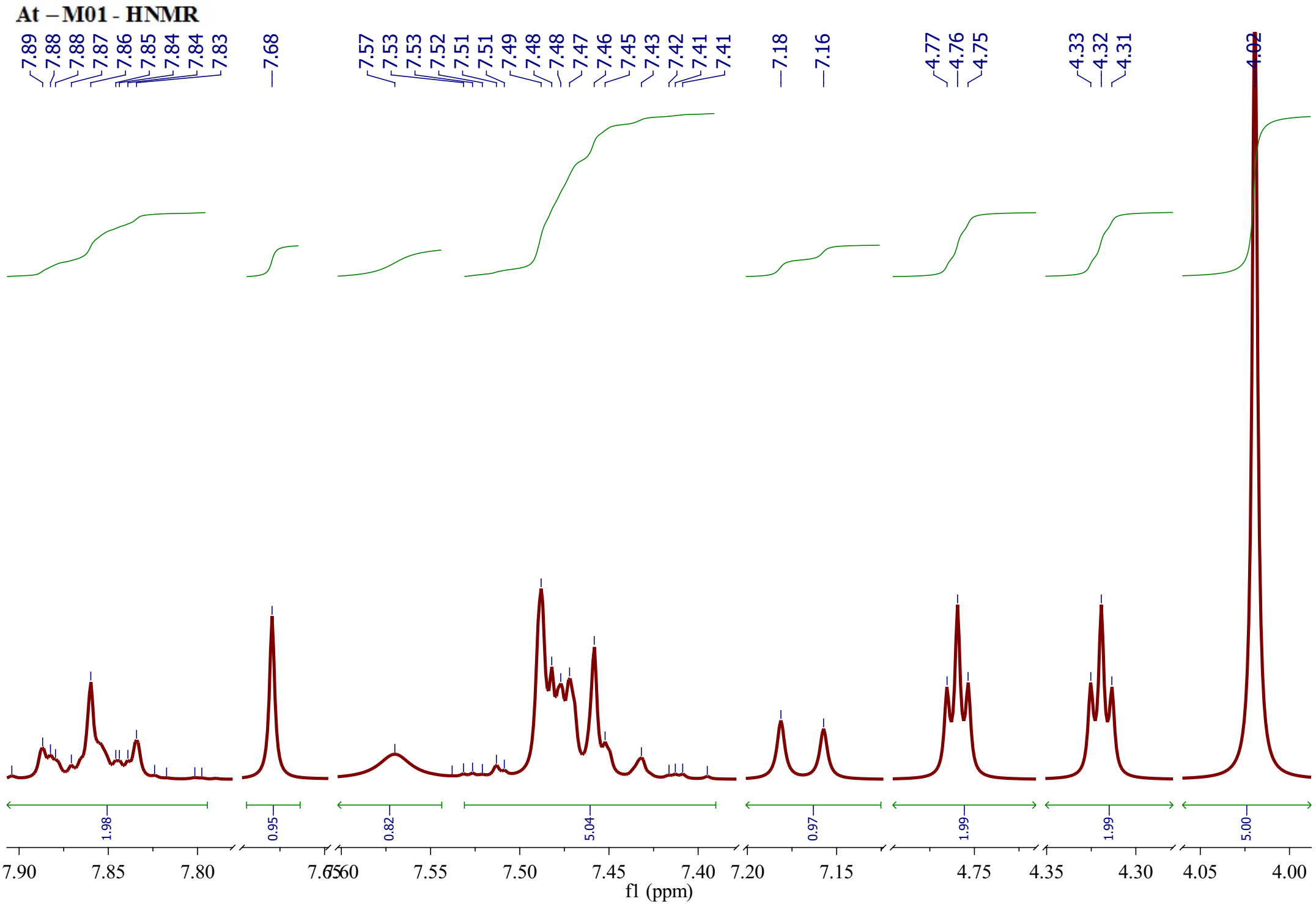

7.73
7.71
7.70
7.69
7.67
7.61
7.60
7.59
7.57
7.55
7.54
7.53
7.51
7.51
7.50
7.49
7.48
7.47
7.47
7.46
7.45
7.32
7.30
7.29
7.28
4.69
4.68
4.67
4.34
4.33
4.32
4.02
3.30
2.50
2.35
2.08
5.29
2.08
1.93
2.03
5.08
3.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

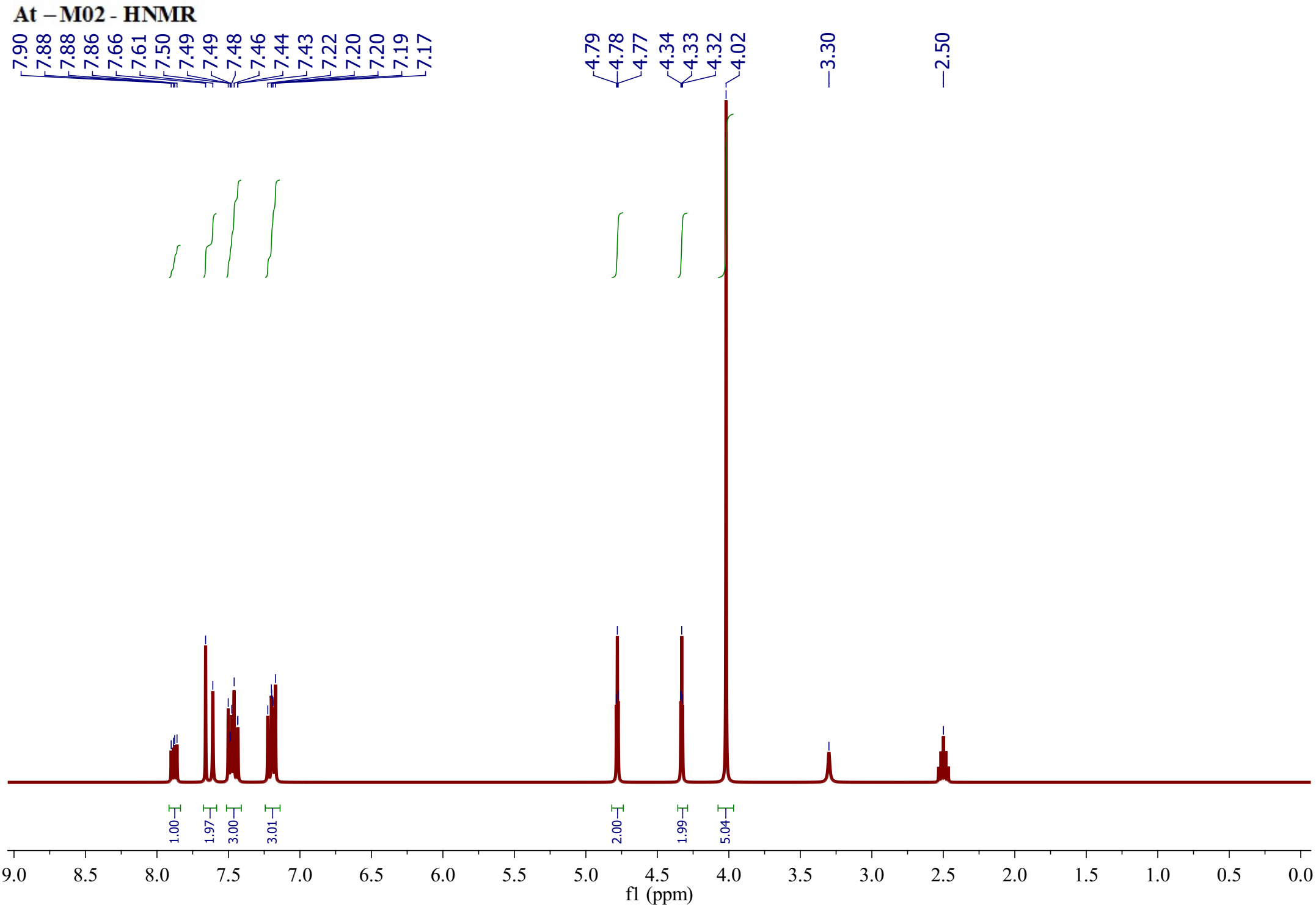
At – P10 - HNMR

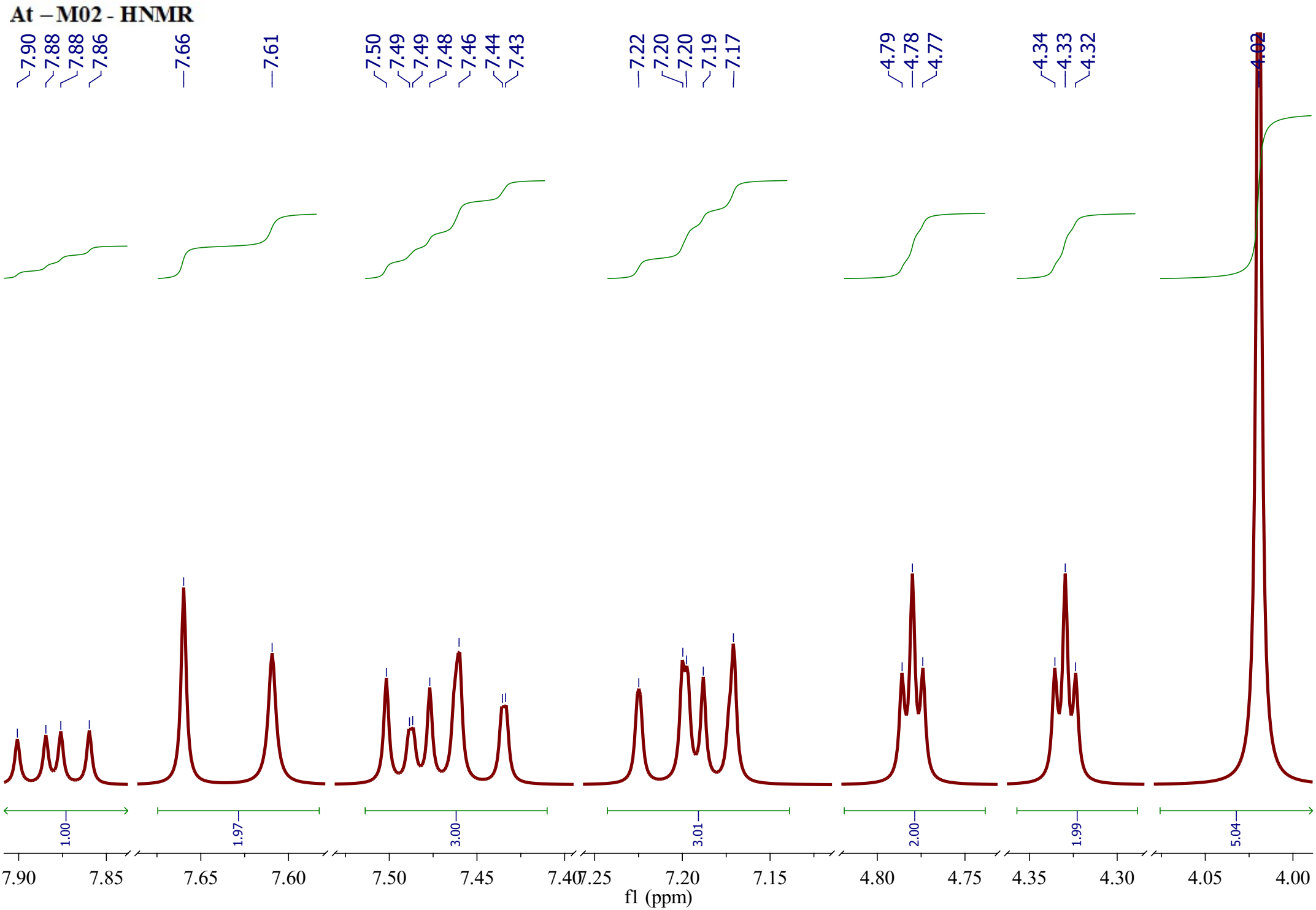

7.73
7.71
7.70
7.69
7.67
7.61
7.60
7.59
7.57
7.55
7.54
7.53
7.51
7.51
7.50
7.49
7.48
7.47
7.47
7.45
7.32
7.30
7.29
7.28
7.26
7.26
4.69
4.68
4.67
4.34
4.33
4.32
4.02
2.35
2.08
5.29
2.08
1.93
2.03
5.08
3.00
7.75
7.70
7.65
7.60
7.55
7.50
7.45
7.30
7.25
4.70
4.65
4.35
4.30
4.00
2.35
f1 (ppm)

At – M01 - HNMR

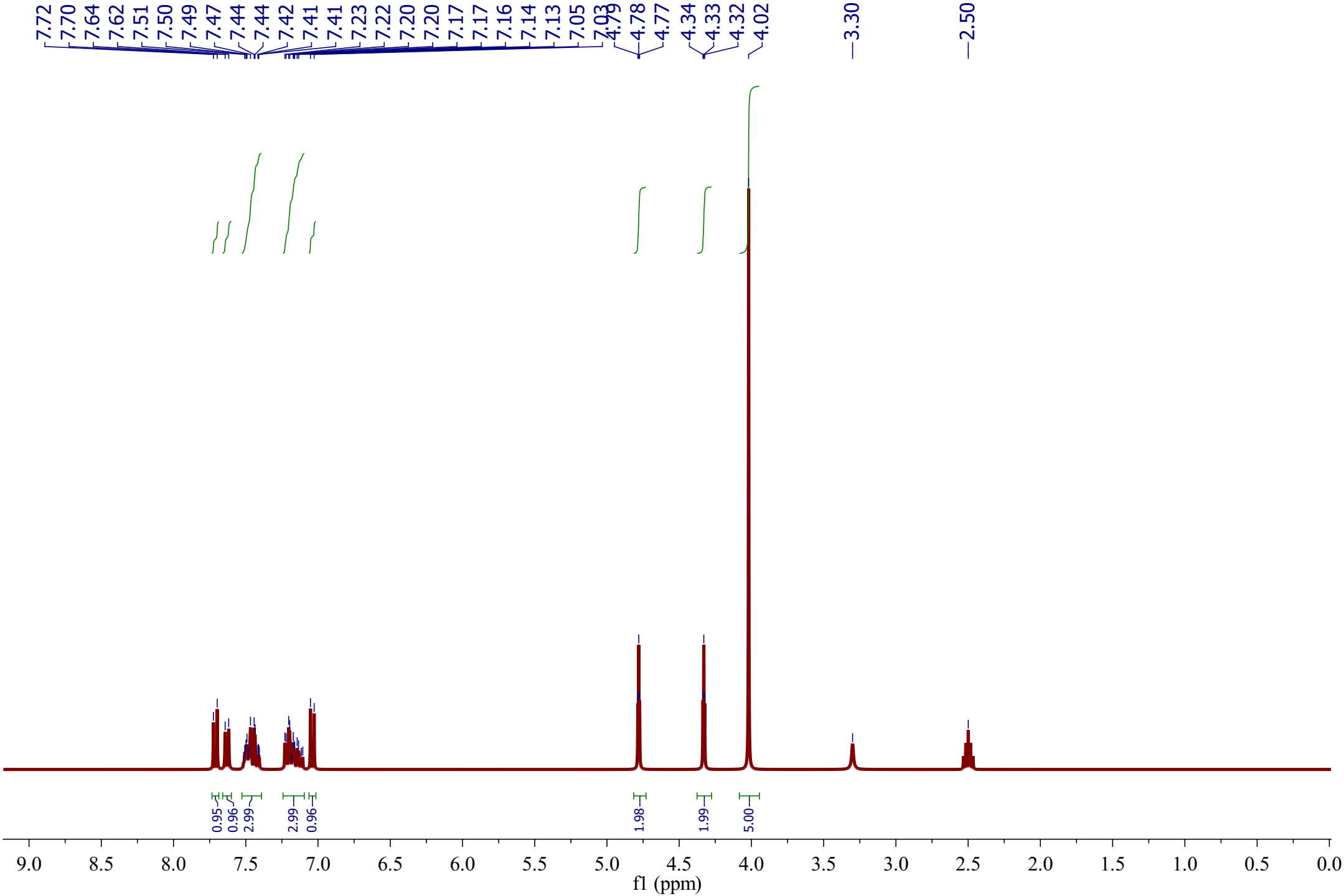

7.89
7.88
7.88
7.87
7.86
7.85
7.84
7.84
7.83
7.68
7.57
7.53
7.53
7.51
7.51
7.49
7.48
7.48
7.47
7.46
7.45
7.43
7.18
7.16
4.77
4.76
4.75
4.33
4.32
4.31
4.02
3.30
2.50
1.98
0.95
0.82
5.04
0.97
1.99
1.99
5.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

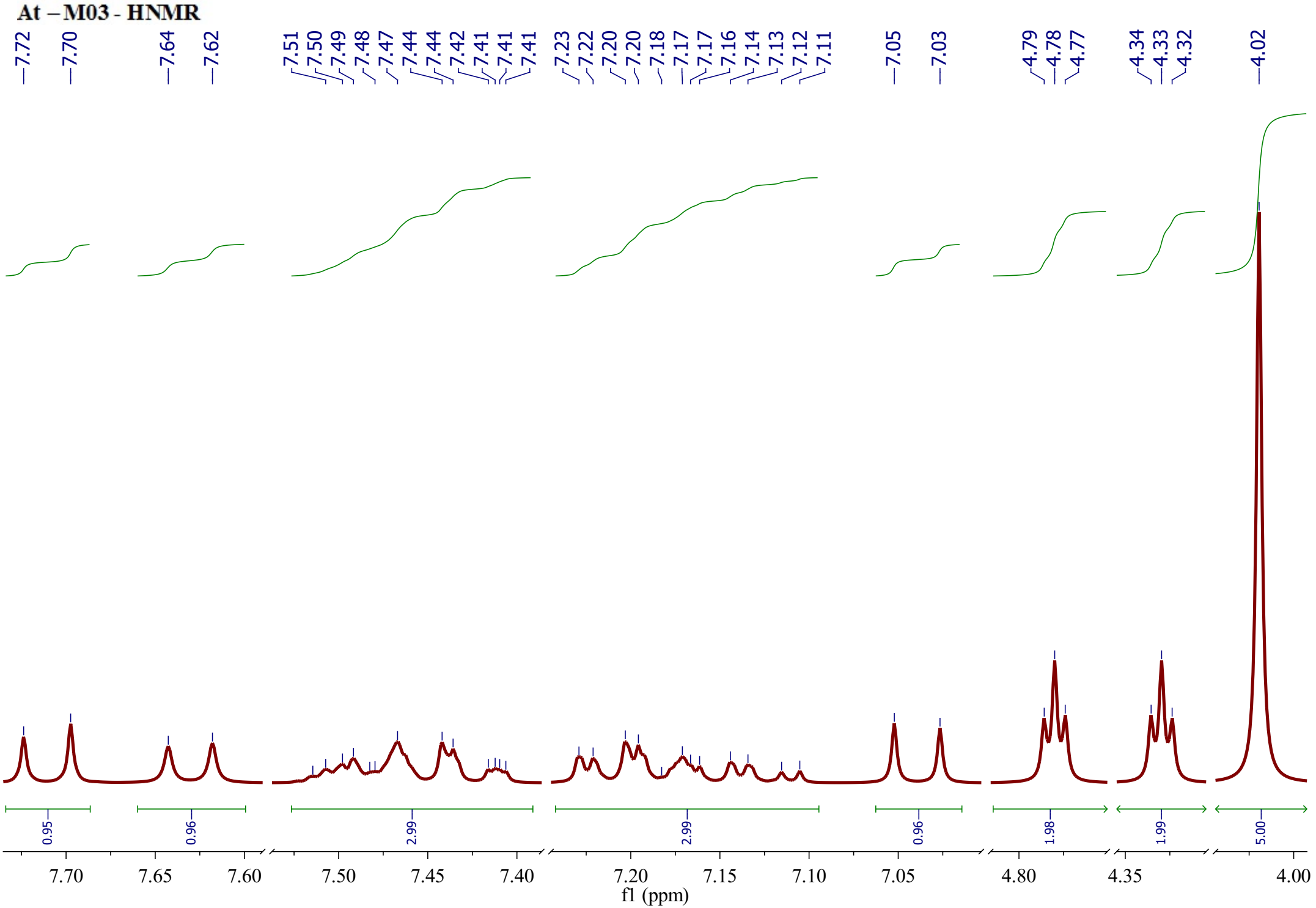

At – M01 - HNMR
7.89
7.88
7.88
7.87
7.86
7.85
7.84
7.84
7.83
7.68
7.57
7.53
7.53
7.52
7.51
7.51
7.49
7.48
7.48
7.47
7.46
7.45
7.43
7.42
7.41
7.41
7.18
7.16
4.77
4.76
4.75
4.33
4.32
4.31
4.02
1.98
0.95
0.82
5.04
0.97
1.99
1.99
5.00
7.90
7.85
7.80
7.65
7.60
7.55
7.50
7.45
7.40
7.20
7.15
4.75
4.35
4.30
4.05
4.00
f1 (ppm)


At – M02 - HNMR
7.90
7.88
7.88
7.86
7.66
7.61
7.50
7.49
7.49
7.48
7.46
7.44
7.43
7.22
7.20
7.20
7.19
7.17
4.79
4.78
4.77
4.34
4.33
4.32
4.02
3.30
2.50
1.00
1.97
3.00
3.01
2.00
1.99
5.04
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

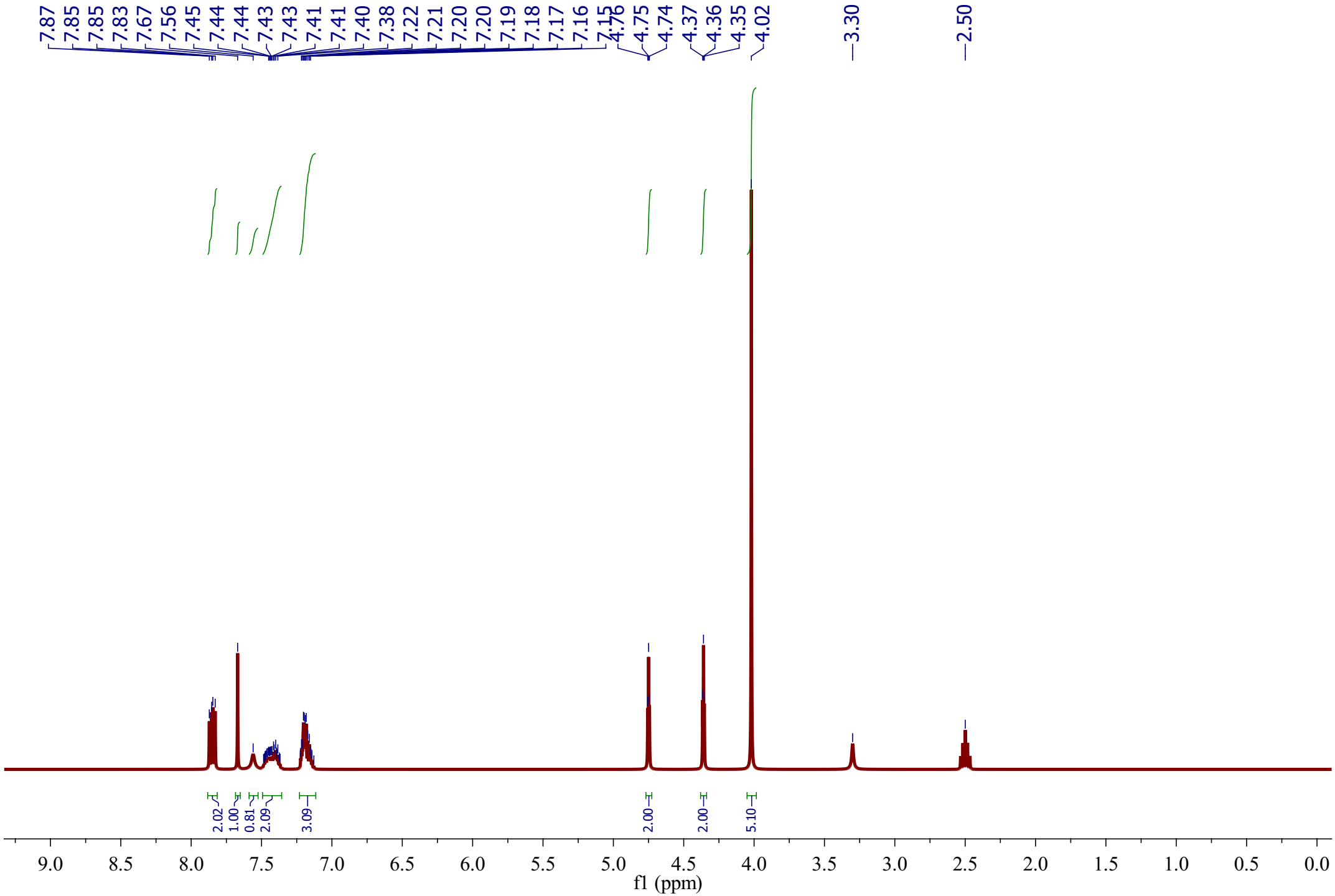

At – M02 - HNMR
7.90
7.88
7.88
7.86
7.66
7.61
7.50
7.49
7.49
7.48
7.46
7.44
7.43
7.22
7.20
7.20
7.19
7.17
4.79
4.78
4.77
4.34
4.33
4.32
4.02
1.00
1.97
3.00
3.01
2.00
1.99
5.04
7.90
7.85
7.65
7.60
7.50
7.45
7.40
7.25
7.20
7.15
4.80
4.75
4.35
4.30
4.05
4.00
f1 (ppm)

At – M03 - HNMR

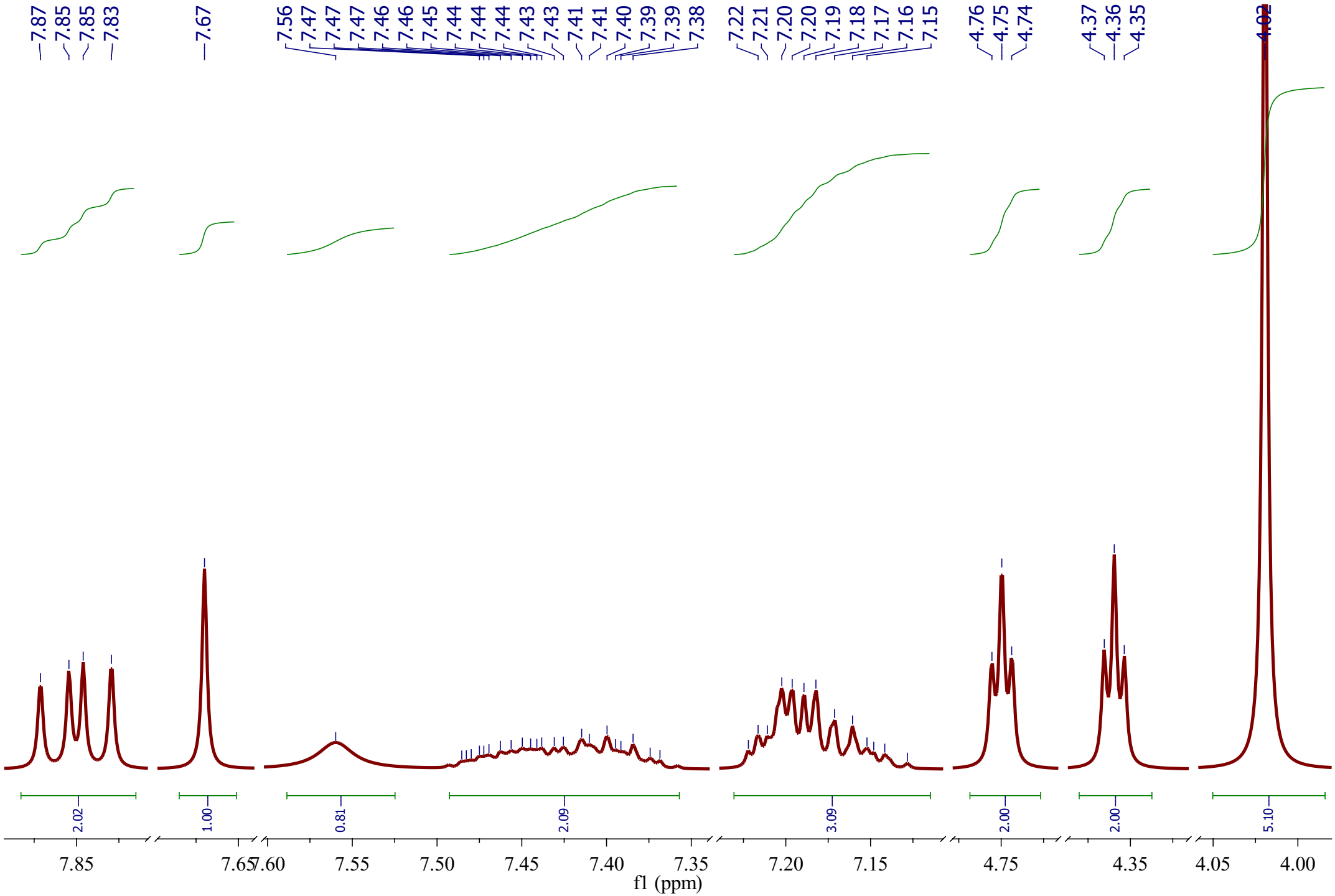

7.72
7.70
7.64
7.62
7.51
7.50
7.49
7.47
7.44
7.44
7.42
7.41
7.41
7.23
7.22
7.20
7.20
7.17
7.17
7.16
7.14
7.13
7.05
7.03
4.79
4.78
4.77
4.34
4.33
4.32
4.02
3.30
2.50
0.95
0.96
2.99
2.99
0.96
1.98
1.99
5.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

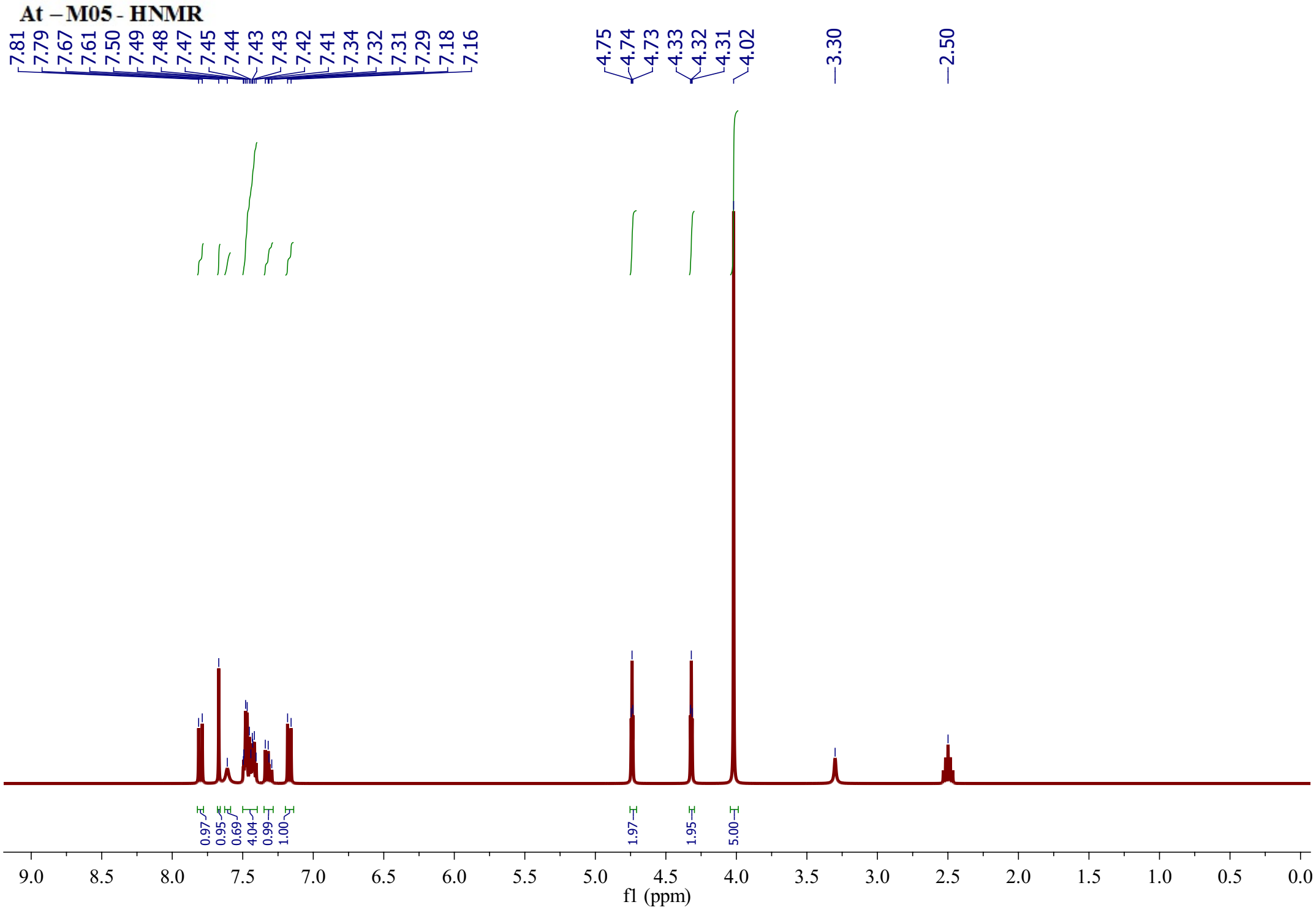

At – M03 - HNMR
7.72
7.70
7.64
7.62
7.51
7.50
7.49
7.48
7.47
7.44
7.44
7.42
7.41
7.41
7.41
7.23
7.22
7.20
7.20
7.18
7.17
7.17
7.16
7.14
7.13
7.12
7.11
7.05
7.03
4.79
4.78
4.77
4.34
4.33
4.32
4.02
0.95
0.96
2.99
2.99
0.96
1.98
1.99
5.00
7.70
7.65
7.60
7.50
7.45
7.40
7.20
7.15
7.10
7.05
4.80
4.35
4.00
f1 (ppm)

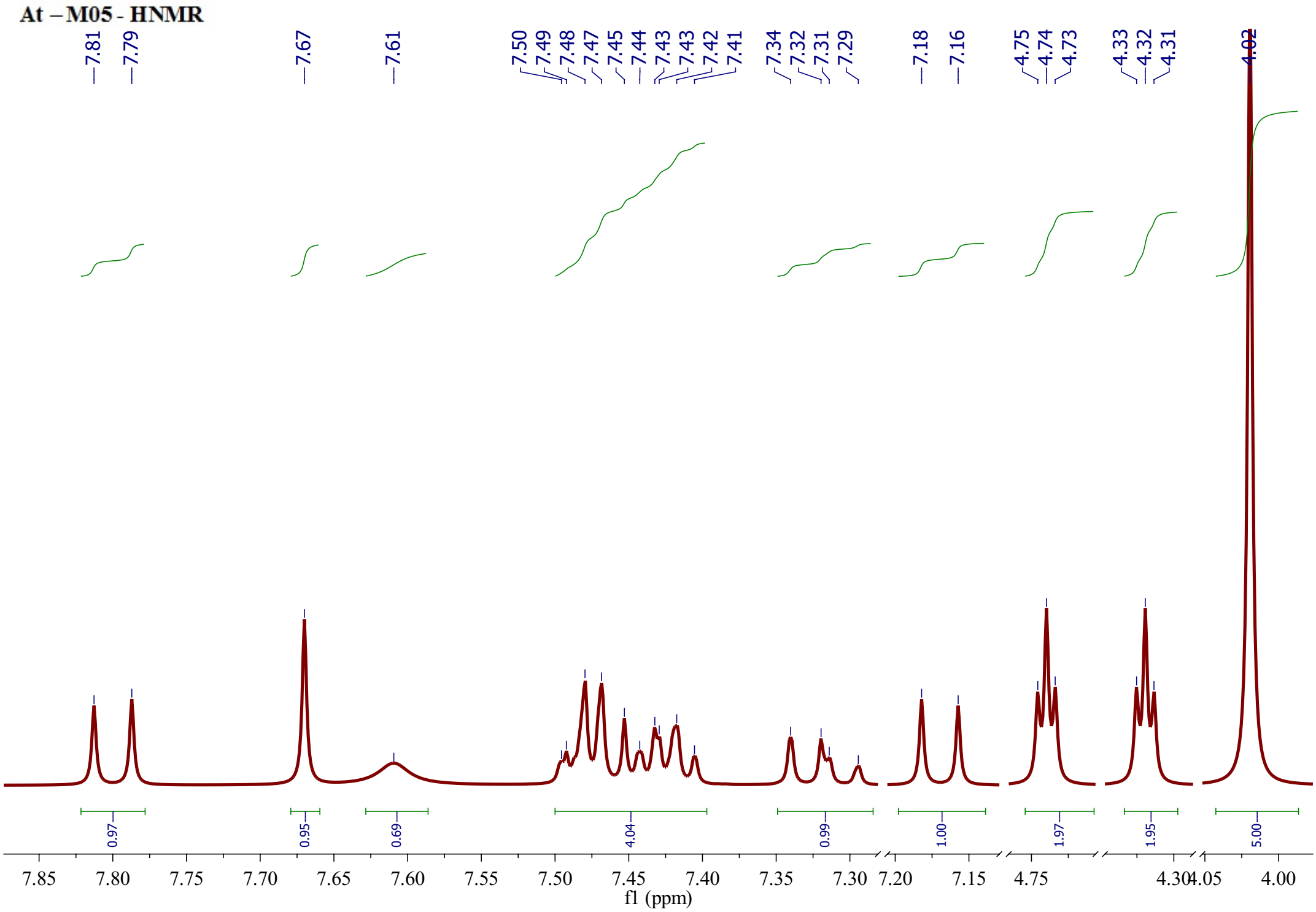
At – M04 - HNMR


7.87
7.85
7.85
7.83
7.67
7.56
7.45
7.44
7.44
7.43
7.43
7.41
7.41
7.40
7.38
7.22
7.21
7.20
7.20
7.19
7.18
7.17
7.16
7.15
4.76
4.75
4.74
4.37
4.36
4.35
4.02
3.30
2.50
2.02
1.00
0.81
2.09
3.09
2.00
2.00
5.10
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

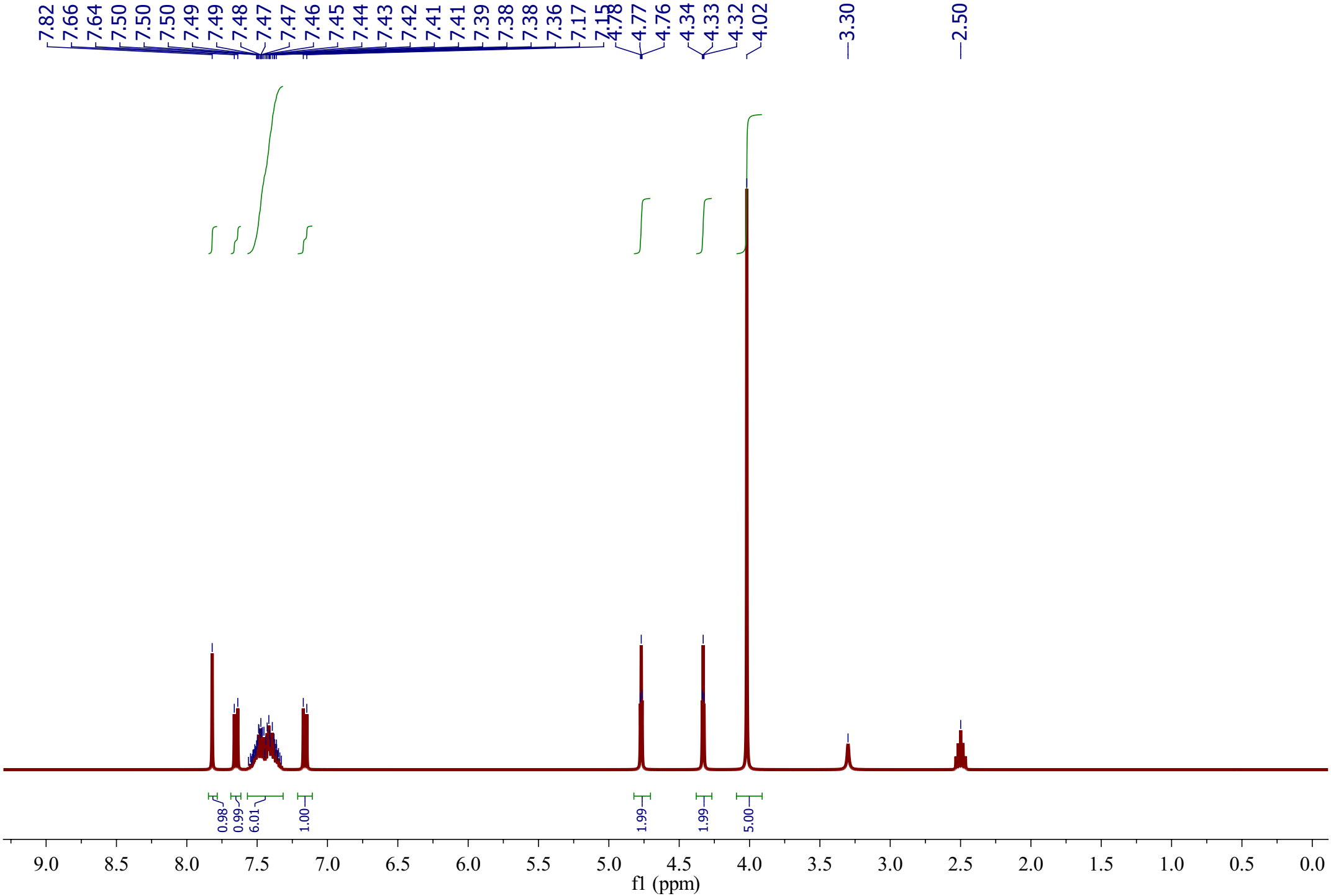
At – M04 - HNMR

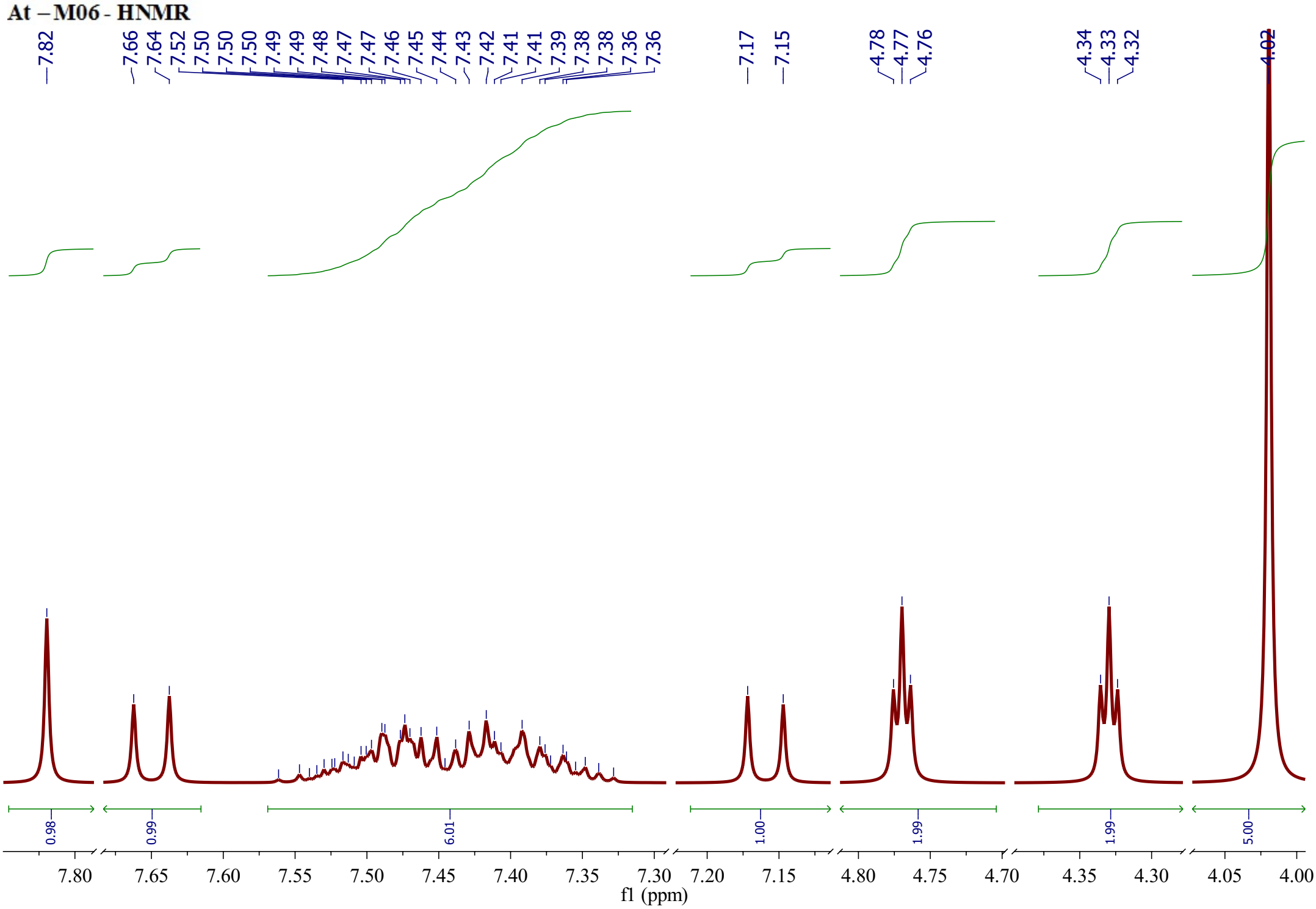

7.87
7.85
7.85
7.83
7.67
7.56
7.47
7.47
7.47
7.46
7.46
7.45
7.44
7.44
7.44
7.43
7.43
7.41
7.41
7.40
7.39
7.39
7.38
7.22
7.21
7.20
7.20
7.19
7.18
7.17
7.16
7.15
4.76
4.75
4.74
4.37
4.36
4.35
4.02
2.02
1.00
0.81
2.09
3.09
2.00
2.00
5.10
7.85
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.20
7.15
4.75
4.35
4.05
4.00
f1 (ppm)


At – M05 - HNMR
7.81
7.79
7.67
7.61
7.50
7.49
7.48
7.47
7.45
7.44
7.43
7.43
7.42
7.41
7.34
7.32
7.31
7.29
7.18
7.16
4.75
4.74
4.73
4.33
4.32
4.31
4.02
3.30
2.50
0.97
0.95
0.69
4.04
0.99
1.00
1.97
1.95
5.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

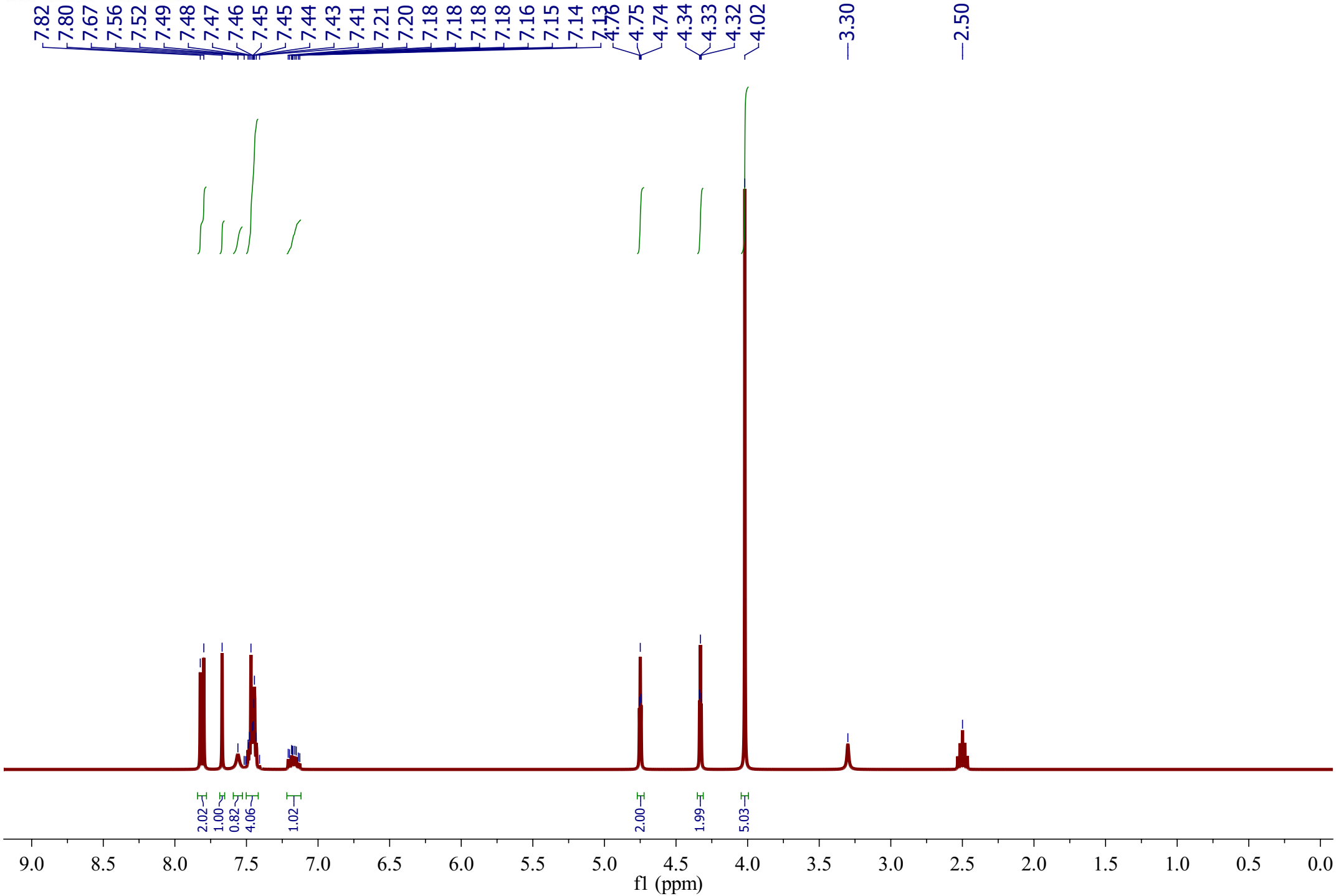

At – M05 - HNMR
7.81
7.79
7.67
7.61
7.50
7.49
7.48
7.47
7.45
7.44
7.43
7.43
7.42
7.41
7.34
7.32
7.31
7.29
7.18
7.16
4.75
4.74
4.73
4.33
4.32
4.31
4.02
0.97
0.95
0.69
4.04
0.99
1.00
1.97
1.95
5.00
7.85
7.80
7.75
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.20
7.15
4.75
4.30
4.05
4.00
f1 (ppm)

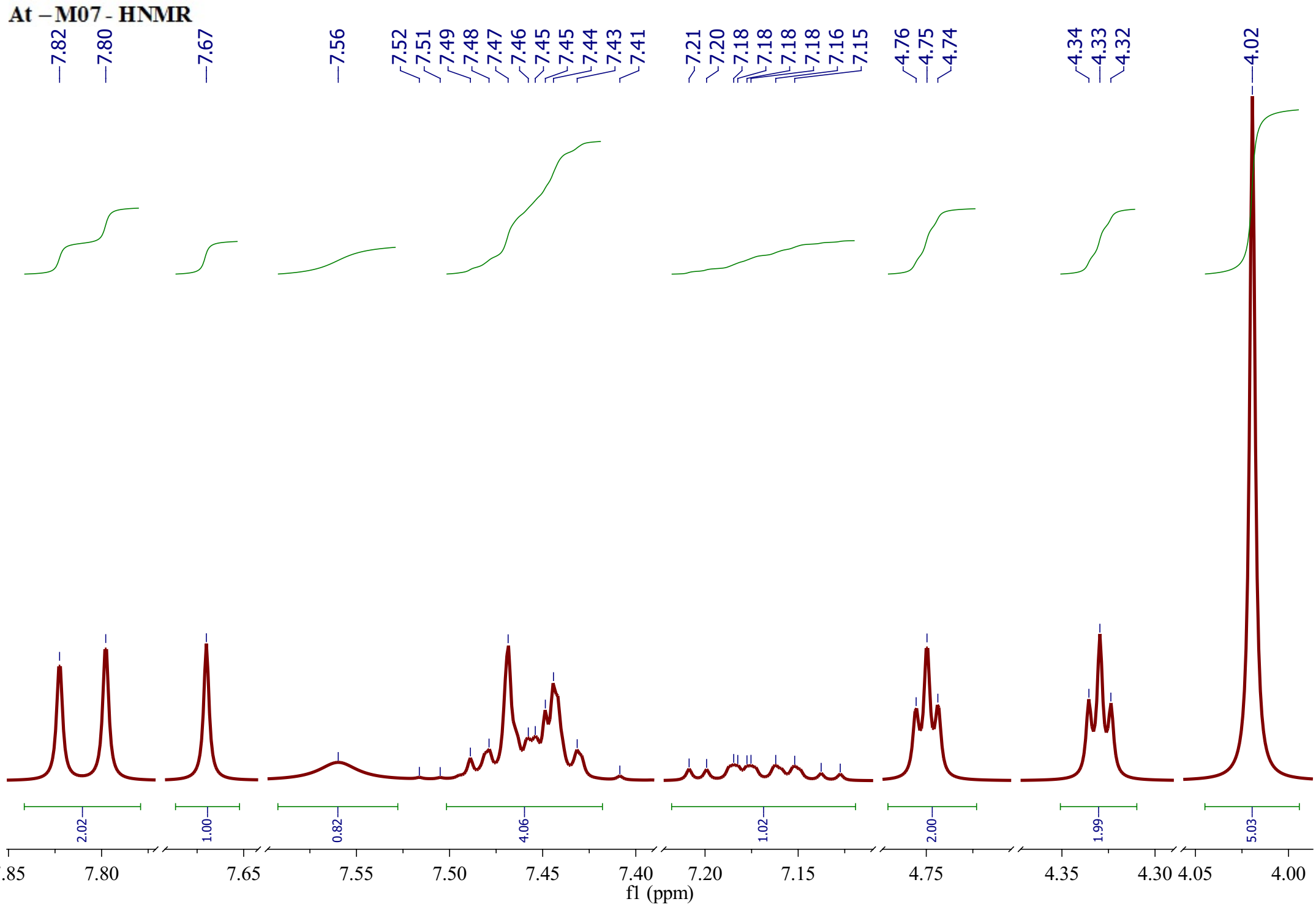
At – M06 - HNMR


7.82
7.66
7.64
7.50
7.50
7.50
7.49
7.49
7.48
7.47
7.47
7.46
7.45
7.44
7.43
7.42
7.41
7.41
7.39
7.38
7.38
7.36
7.17
7.15
4.78
4.77
4.76
4.34
4.33
4.32
4.02
3.30
2.50
0.98
0.99
6.01
1.00
1.99
1.99
5.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

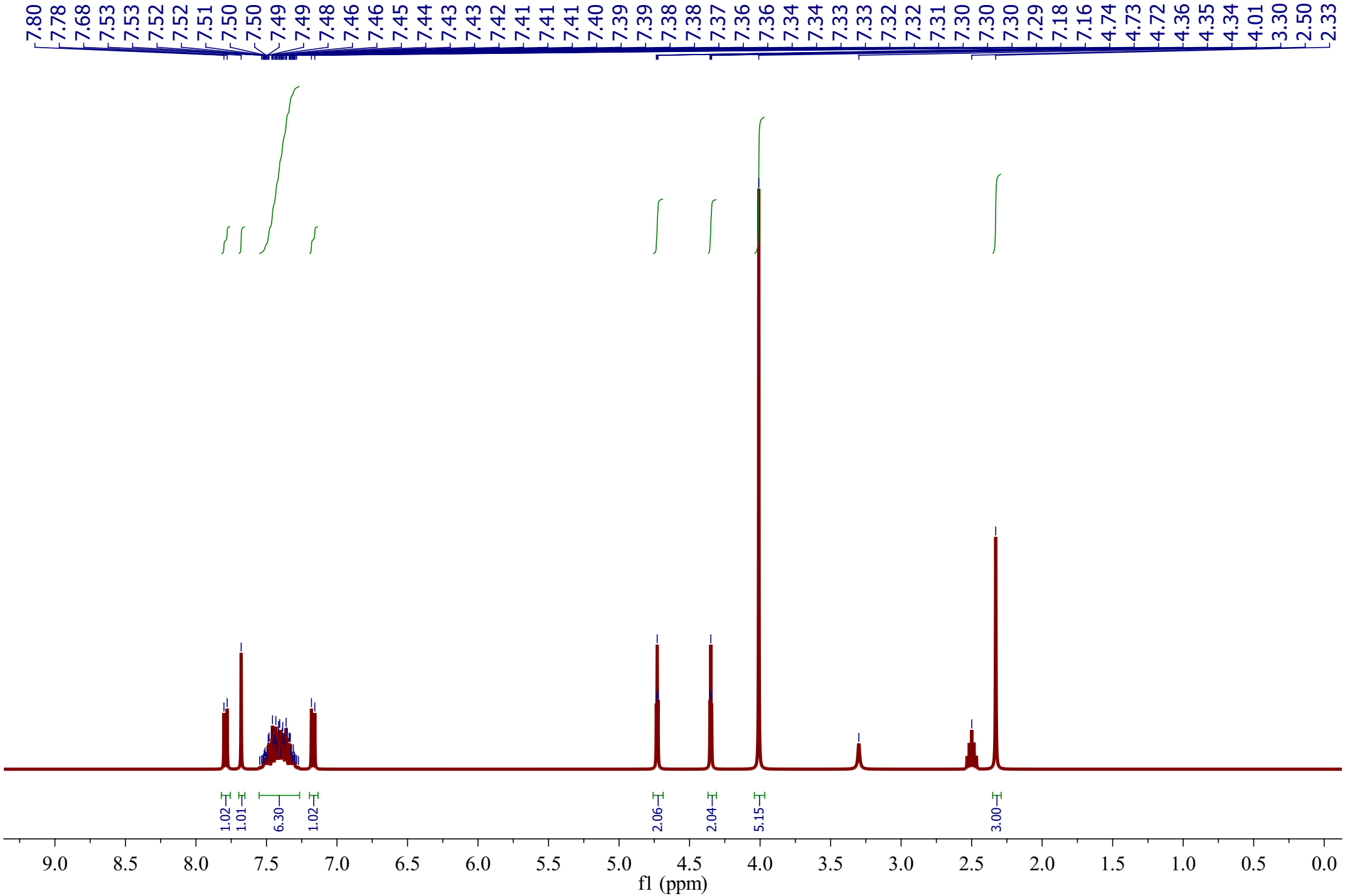

At – M06 - HNMR
7.82
7.66
7.64
7.52
7.50
7.50
7.50
7.49
7.49
7.48
7.47
7.47
7.46
7.45
7.44
7.43
7.42
7.41
7.41
7.39
7.38
7.38
7.36
7.36
7.17
7.15
4.78
4.77
4.76
4.34
4.33
4.32
4.02
0.98
0.99
6.01
1.00
1.99
1.99
5.00
7.80
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.20
7.15
4.80
4.75
4.70
4.35
4.30
4.05
4.00
f1 (ppm)

At – M07 - HNMR

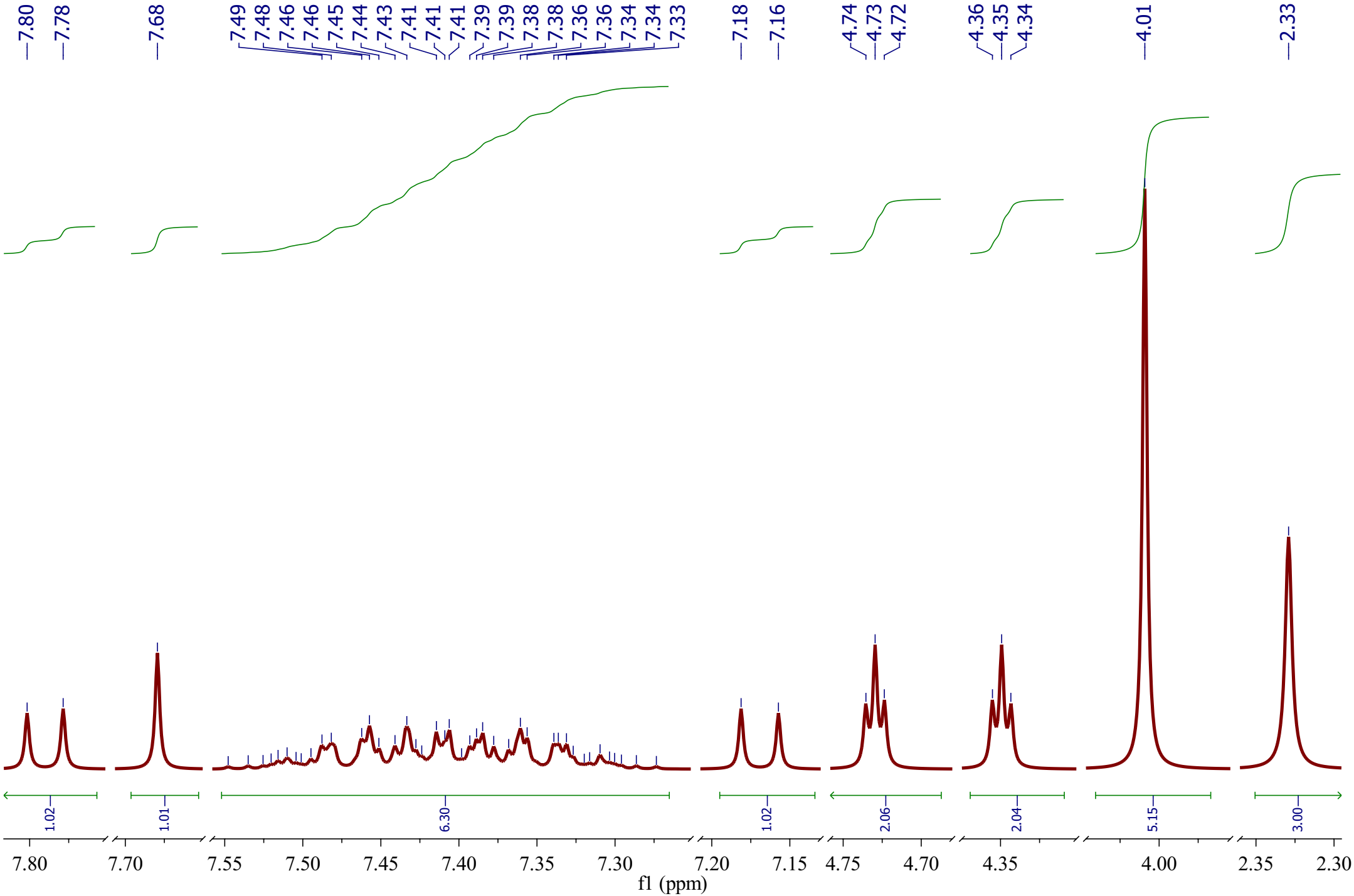

7.82
7.80
7.67
7.56
7.52
7.49
7.48
7.47
7.46
7.45
7.45
7.44
7.43
7.41
7.21
7.20
7.18
7.18
7.18
7.16
7.15
7.14
7.13
4.76
4.75
4.74
4.34
4.33
4.32
4.02
3.30
2.50
2.02
1.00
0.82
4.06
1.02
2.00
1.99
5.03
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


At – M07 - HNMR
7.82
7.80
7.67
7.56
7.52
7.51
7.49
7.48
7.47
7.46
7.45
7.45
7.44
7.43
7.41
7.21
7.20
7.18
7.18
7.18
7.18
7.16
7.15
4.76
4.75
4.74
4.34
4.33
4.32
4.02
2.02
1.00
0.82
4.06
1.02
2.00
1.99
5.03
7.85
7.80
7.65
7.55
7.50
7.45
7.40
7.20
7.15
4.75
4.35
4.30
4.05
4.00
f1 (ppm)

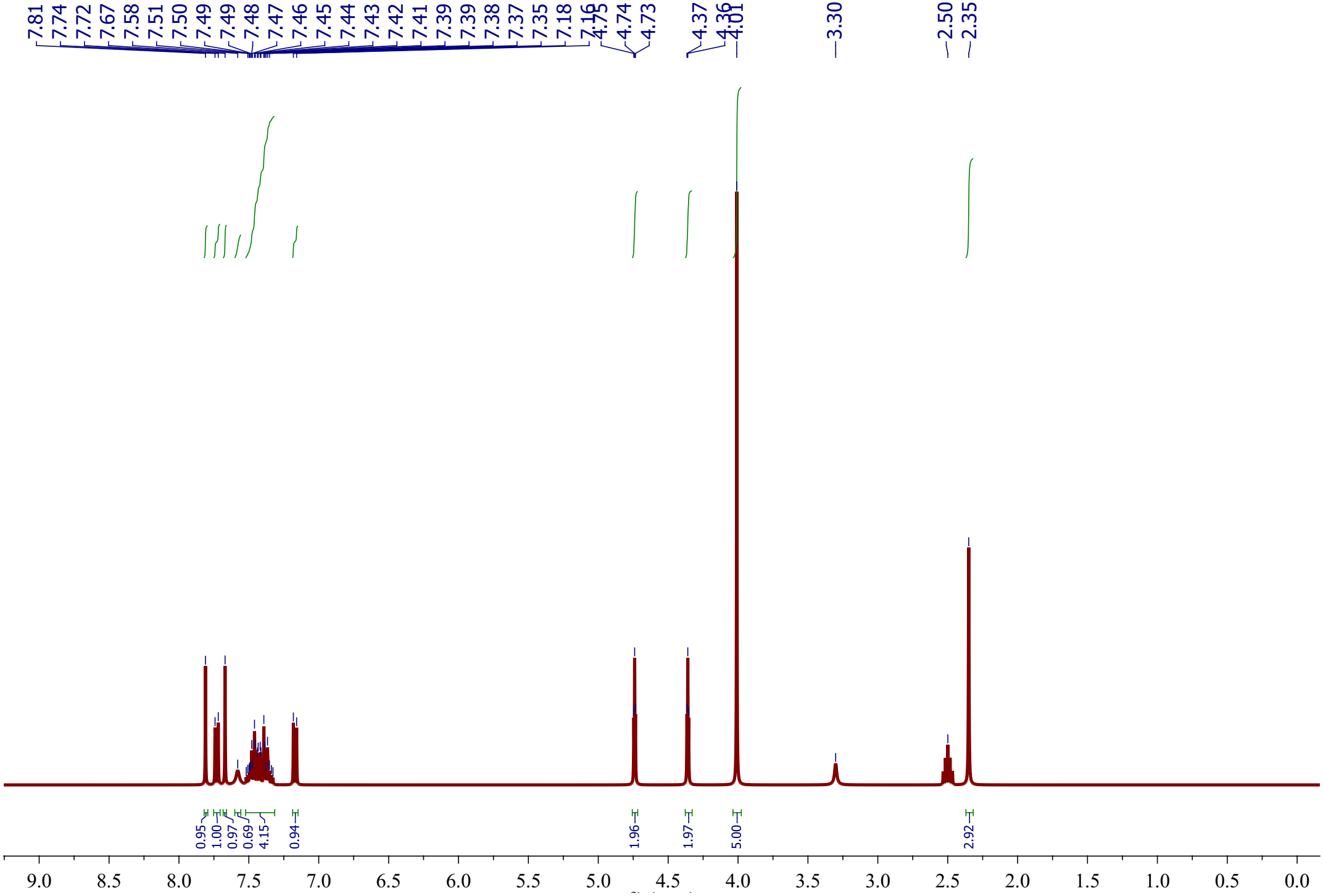
At – M08 - HNMR


7.80 7.78 7.68 7.53 7.53 7.52 7.52 7.51 7.50 7.50 7.49 7.49 7.48 7.46 7.46 7.45 7.44 7.43 7.43 7.42 7.41 7.41 7.41 7.40 7.39 7.39 7.38 7.38 7.37 7.36 7.36 7.34 7.34 7.33 7.33 7.32 7.32 7.31 7.30 7.30 7.30 7.29 7.18 7.16 4.74 4.73 4.72 4.36 4.35 4.34 4.01 3.30 2.50 2.33
1.02 1.01 6.30 1.02 2.06 2.04 5.15 3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

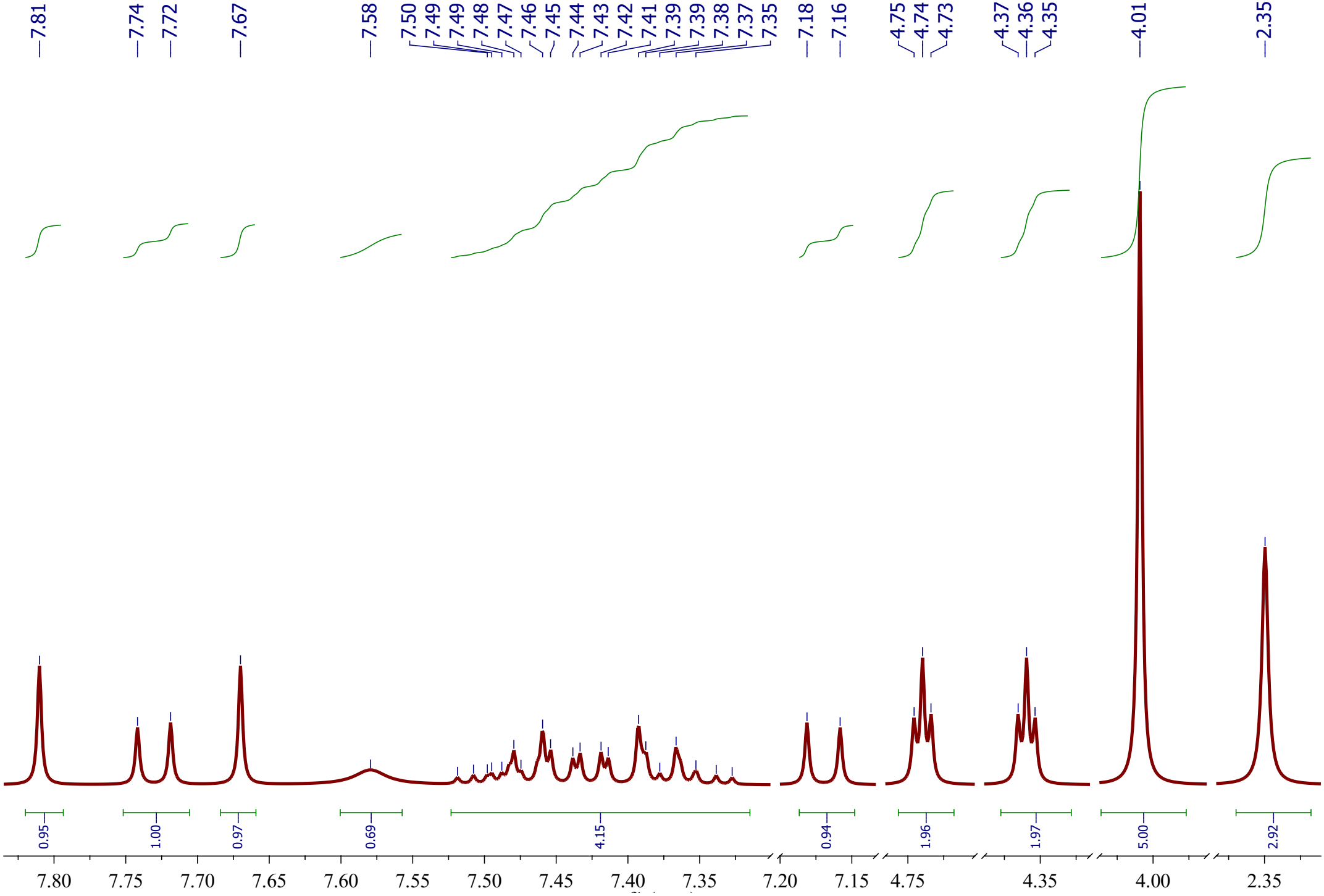
At – M08 - HNMR

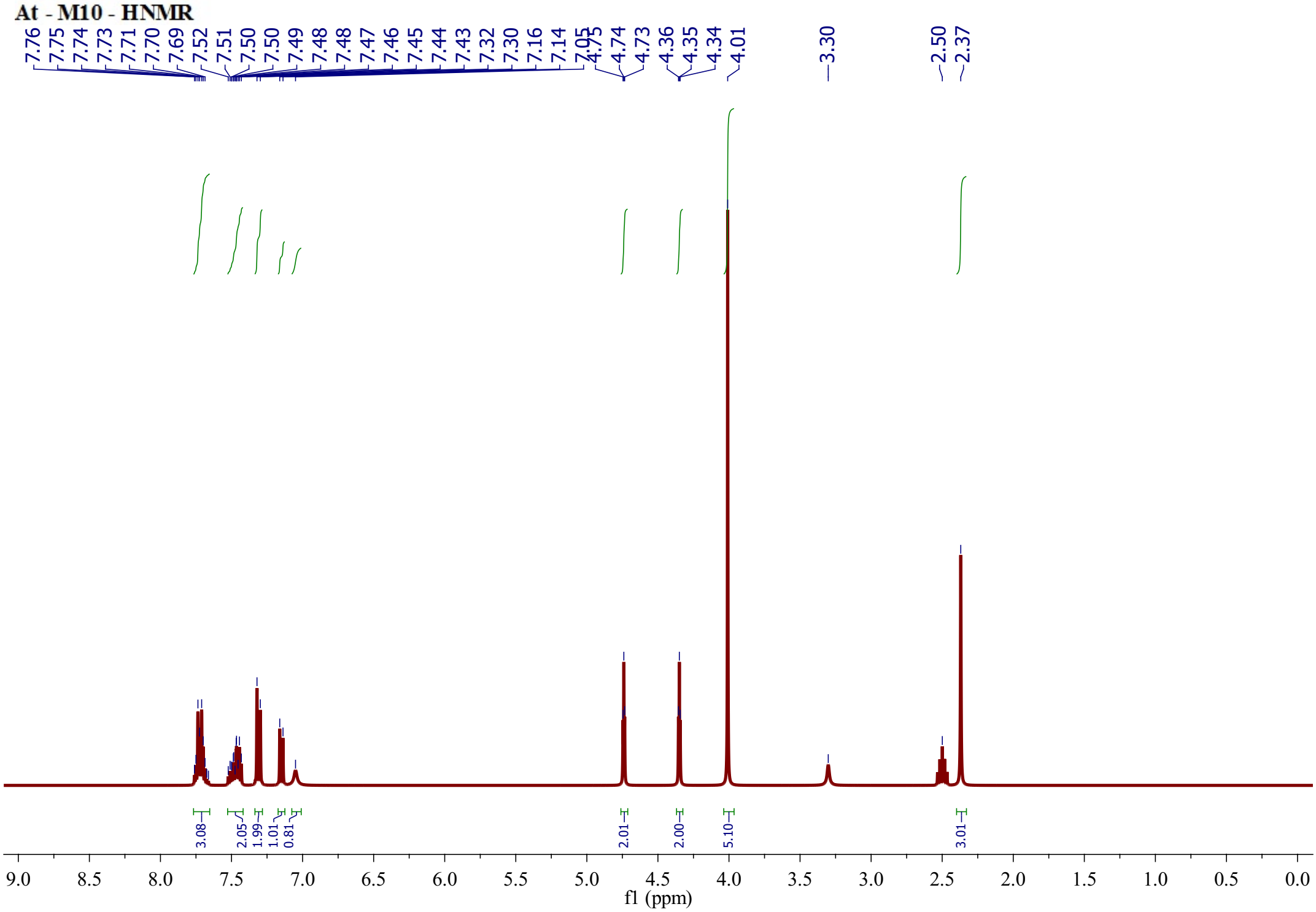

7.80
7.78
7.68
7.49
7.48
7.46
7.46
7.45
7.44
7.43
7.41
7.41
7.41
7.39
7.39
7.38
7.38
7.36
7.36
7.34
7.34
7.33
7.18
7.16
4.74
4.73
4.72
4.36
4.35
4.34
4.01
2.33
1.02
1.01
6.30
1.02
2.06
2.04
5.15
3.00
7.80
7.70
7.55
7.50
7.45
7.40
7.35
7.30
7.20
7.15
4.75
4.70
4.35
4.00
2.35
2.30
f1 (ppm)

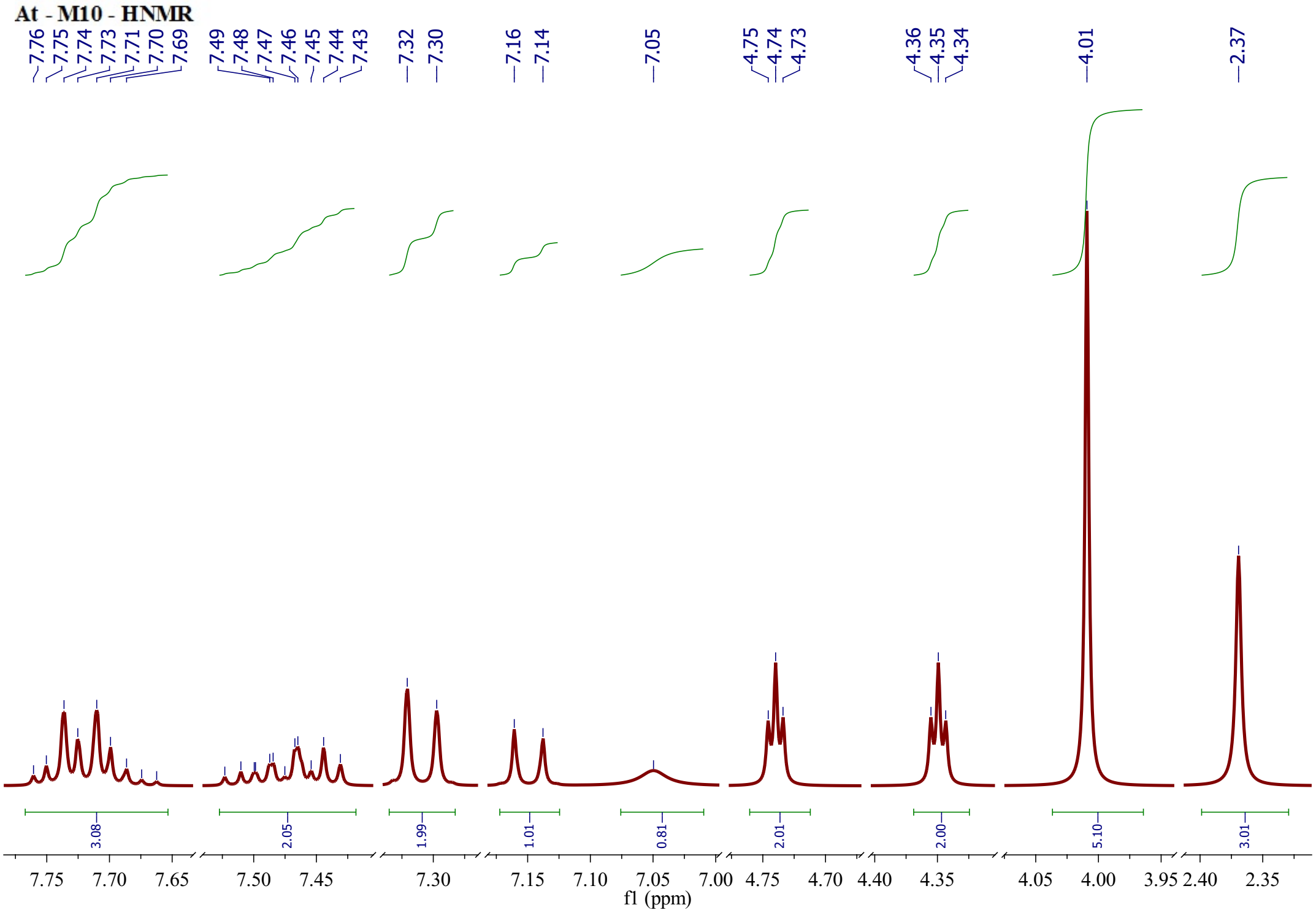
At – M09 - HNMR

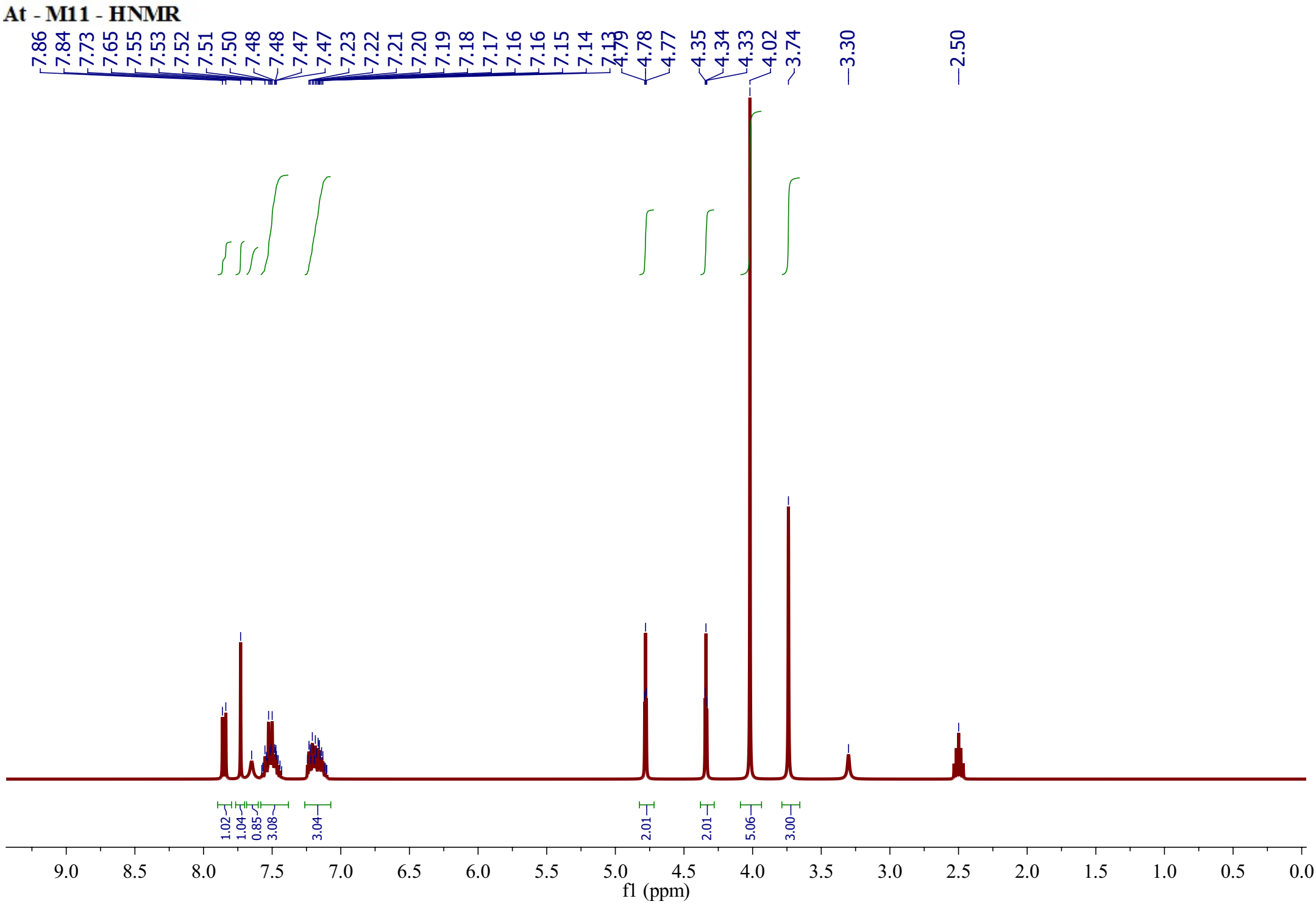

7.81
7.74
7.72
7.67
7.58
7.51
7.50
7.49
7.49
7.48
7.47
7.46
7.45
7.44
7.43
7.42
7.41
7.39
7.39
7.38
7.37
7.35
7.18
7.16
4.75
4.74
4.73
4.37
4.36
4.01
3.30
2.50
2.35
0.95
1.00
0.97
0.69
4.15
0.94
1.96
1.97
5.00
2.92
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

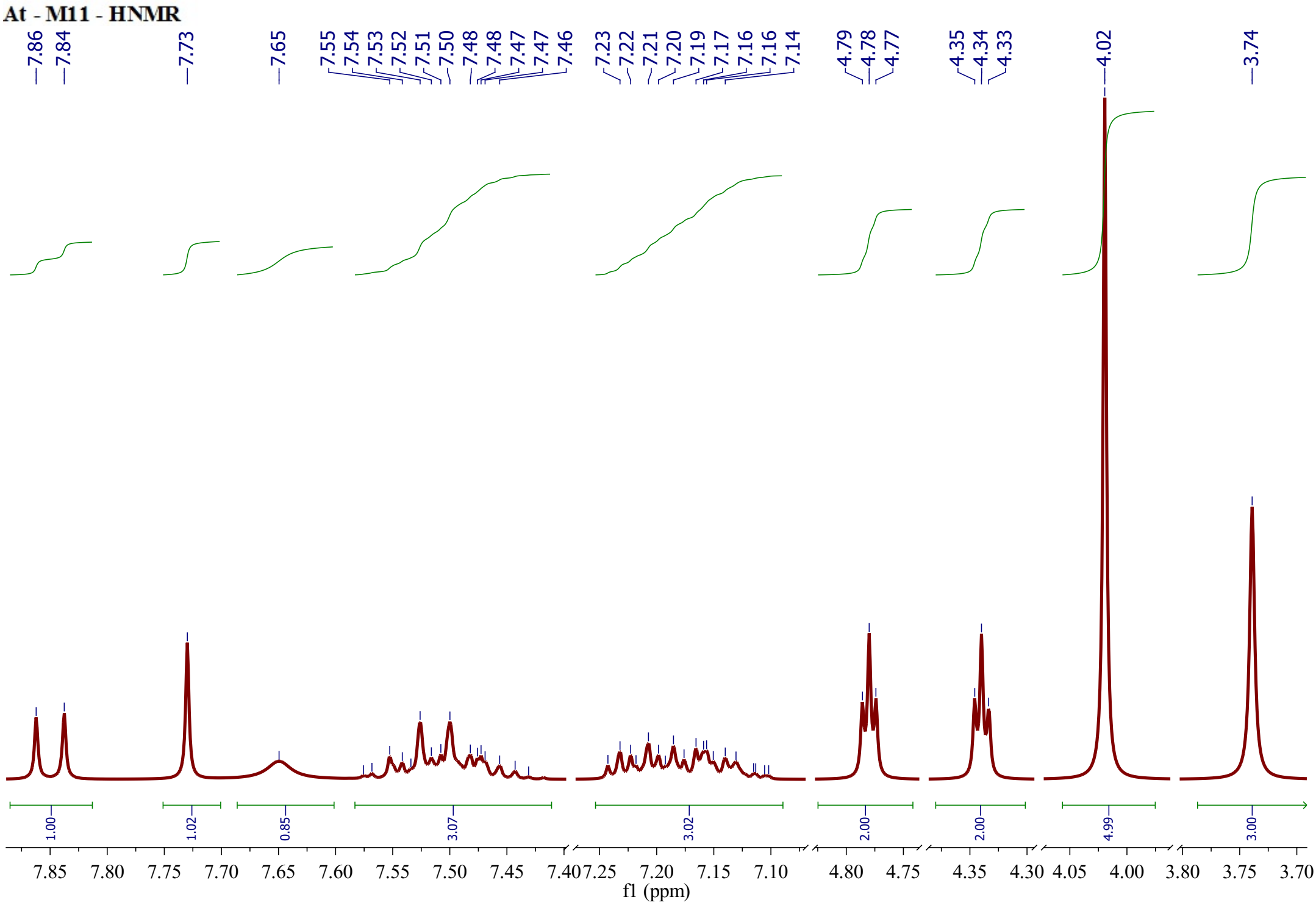
At – M09 - HNMR


7.81
7.74
7.72
7.67
7.58
7.50
7.49
7.49
7.48
7.47
7.46
7.45
7.44
7.43
7.42
7.41
7.39
7.39
7.38
7.37
7.35
7.18
7.16
4.75
4.74
4.73
4.37
4.36
4.35
4.01
2.35
0.95
1.00
0.97
0.69
4.15
0.94
1.96
1.97
5.00
2.92
7.80
7.75
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.20
7.15
4.75
4.35
4.00
2.35

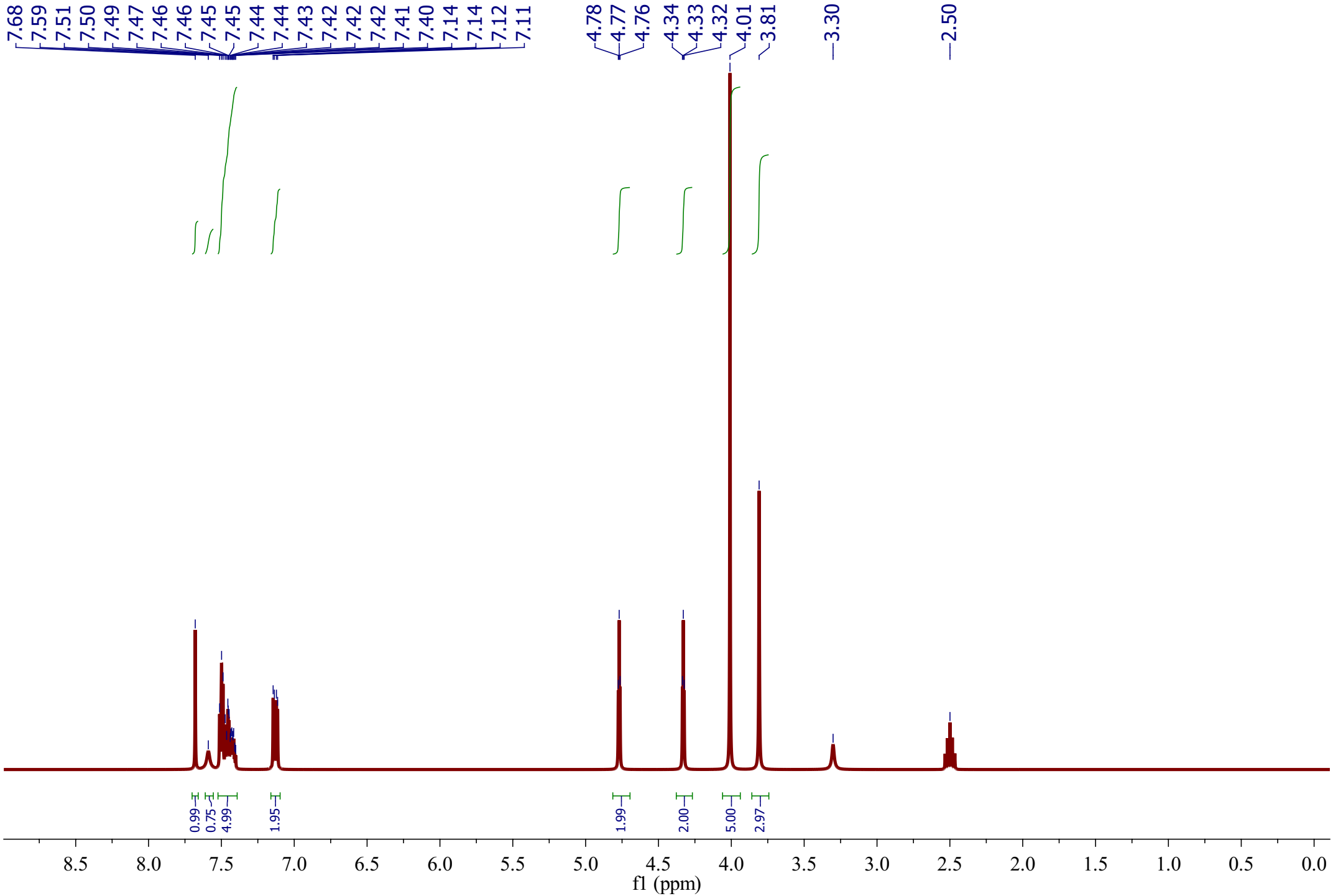

At - M10 - HNMR
7.76
7.75
7.74
7.73
7.71
7.70
7.69
7.52
7.51
7.50
7.50
7.49
7.48
7.48
7.47
7.46
7.45
7.44
7.43
7.32
7.30
7.16
7.14
7.05
4.75
4.74
4.73
4.36
4.35
4.34
4.01
3.30
2.50
2.37
3.08
2.05
1.99
1.01
0.81
2.01
2.00
5.10
3.01
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


At - M10 - HNMR
7.76
7.75
7.74
7.73
7.71
7.70
7.69
7.49
7.48
7.47
7.46
7.45
7.44
7.43
7.32
7.30
7.16
7.14
7.05
4.75
4.74
4.73
4.36
4.35
4.34
4.01
2.37
3.08
2.05
1.99
1.01
0.81
2.01
2.00
5.10
3.01
7.75
7.70
7.65
7.50
7.45
7.30
7.15
7.10
7.05
7.00
4.75
4.70
4.40
4.35
4.05
4.00
3.95
2.40
2.35
f1 (ppm)

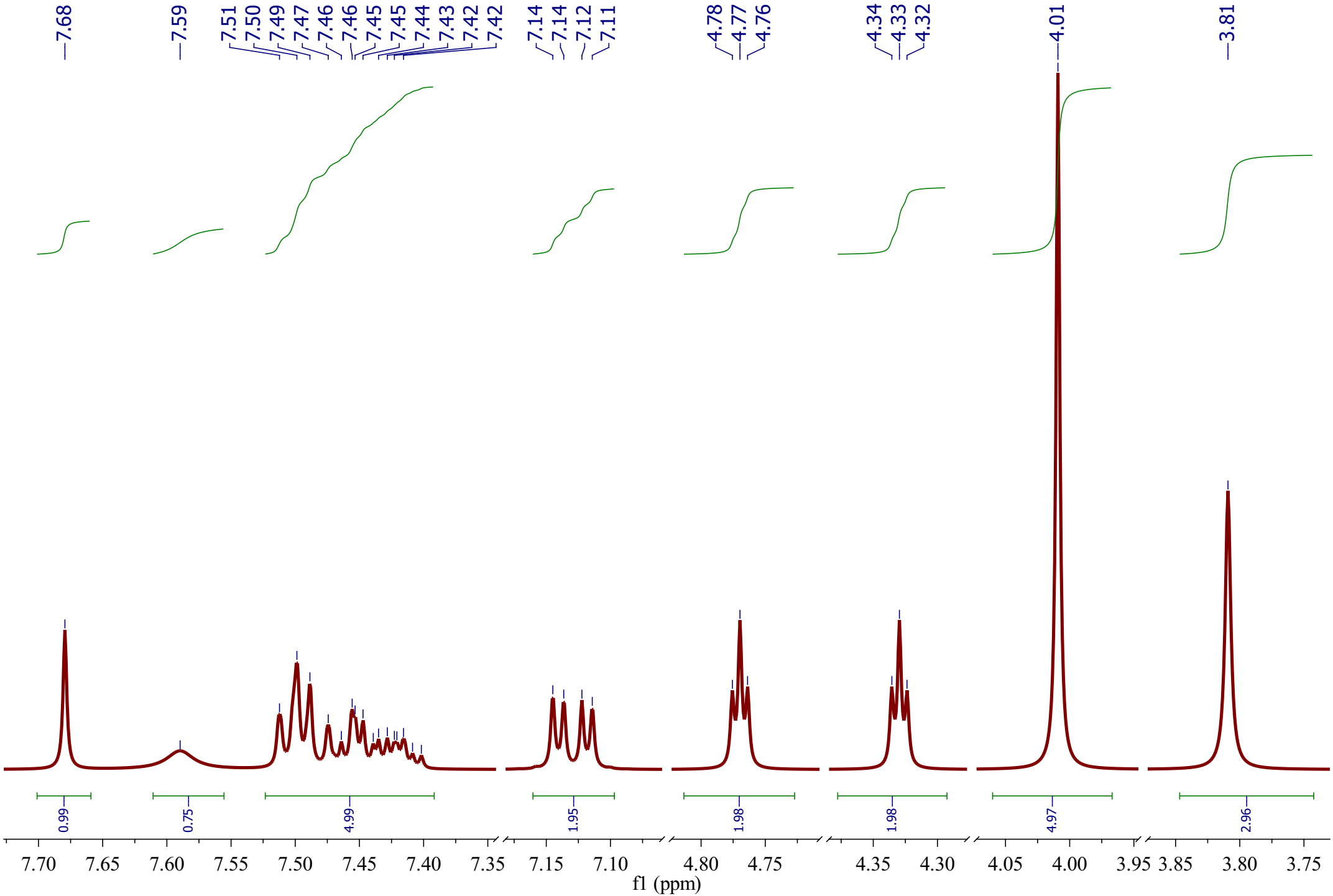

At - M11 - HNMR
7.86
7.84
7.73
7.65
7.55
7.53
7.52
7.51
7.50
7.48
7.48
7.47
7.47
7.23
7.22
7.21
7.20
7.19
7.18
7.17
7.16
7.16
7.15
7.14
7.13
4.79
4.78
4.77
4.35
4.34
4.33
4.02
3.74
3.30
2.50
1.02
1.04
0.85
3.08
3.04
2.01
2.01
5.06
3.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

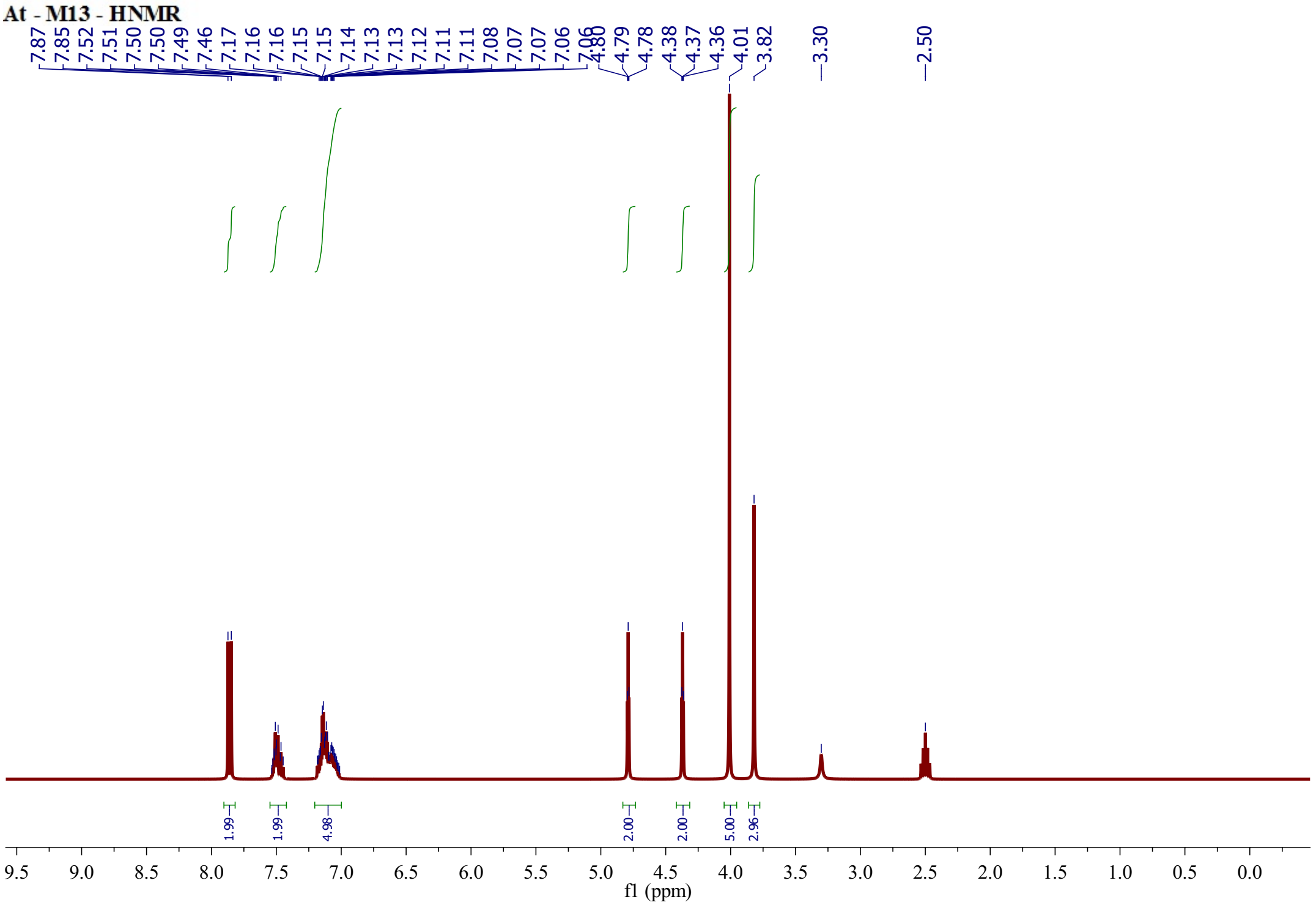

At - M11 - HNMR
7.86
7.84
7.73
7.65
7.55
7.54
7.53
7.52
7.51
7.50
7.48
7.48
7.47
7.47
7.46
7.23
7.22
7.21
7.20
7.19
7.17
7.16
7.16
7.14
4.79
4.78
4.77
4.35
4.34
4.33
4.02
3.74
1.00
1.02
0.85
3.07
3.02
2.00
2.00
4.99
3.00
7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.25 7.20 7.15 7.10 4.80 4.75 4.35 4.30 4.05 4.00 3.80 3.75 3.70
f1 (ppm)

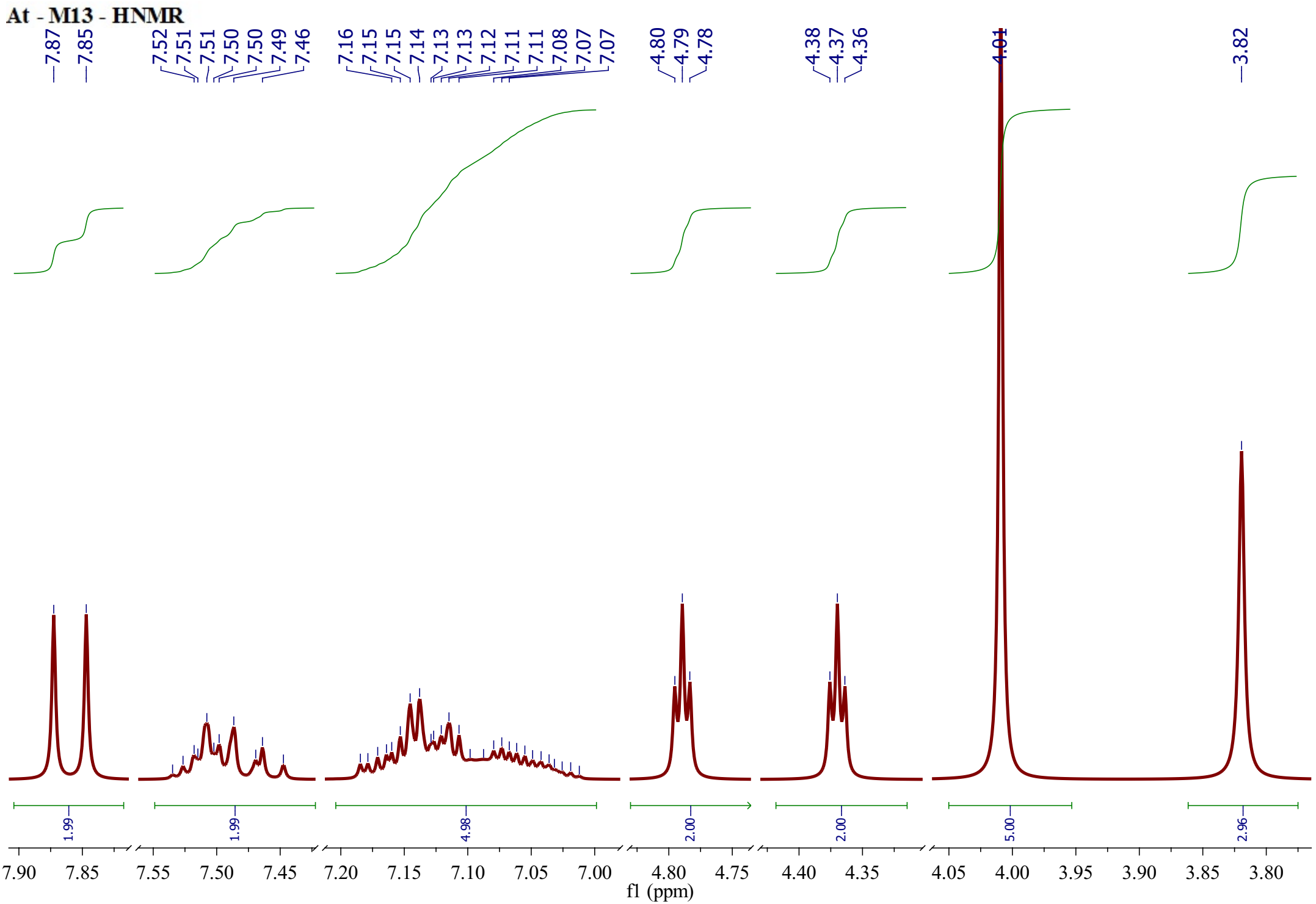
At - M12 - HNMR


7.68
7.59
7.51
7.50
7.49
7.47
7.46
7.46
7.45
7.45
7.44
7.44
7.43
7.42
7.42
7.42
7.41
7.40
7.14
7.14
7.12
7.11
4.78
4.77
4.76
4.34
4.33
4.32
4.01
3.81
3.30
2.50
0.99
0.75
4.99
1.95
1.99
2.00
5.00
2.97
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

At - M12 - HNMR


7.68
7.59
7.51
7.50
7.49
7.47
7.46
7.46
7.45
7.45
7.44
7.43
7.42
7.42
7.14
7.14
7.12
7.11
4.78
4.77
4.76
4.34
4.33
4.32
4.01
3.81
0.99
0.75
4.99
1.95
1.98
1.98
4.97
2.96
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.15
7.10
4.80
4.75
4.35
4.30
4.05
4.00
3.95
3.85
3.80
3.75
f1 (ppm)


At - M13 - HNMR
7.87
7.85
7.52
7.51
7.50
7.50
7.49
7.46
7.17
7.16
7.16
7.15
7.15
7.14
7.13
7.13
7.12
7.11
7.11
7.08
7.07
7.07
7.06
7.06
4.80
4.79
4.78
4.38
4.37
4.36
4.01
3.82
3.30
2.50
1.99
1.99
4.98
2.00
2.00
5.00
2.96
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


At - M13 - HNMR
7.87
7.85
7.52
7.51
7.51
7.50
7.50
7.49
7.46
7.16
7.15
7.15
7.14
7.13
7.13
7.12
7.11
7.11
7.08
7.07
7.07
4.80
4.79
4.78
4.38
4.37
4.36
4.01
3.82
1.99
1.99
4.98
2.00
2.00
5.00
2.96
7.90
7.85
7.55
7.50
7.45
7.20
7.15
7.10
7.05
7.00
4.80
4.75
4.40
4.35
4.05
4.00
3.95
3.90
3.85
3.80
f1 (ppm)

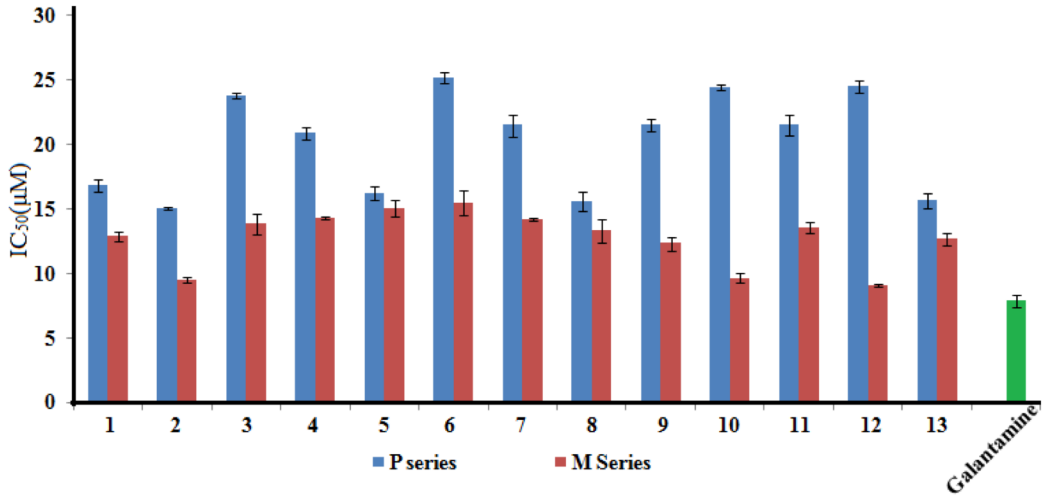


Figure 4: *In-vitro* IC_{50} (µM) values chart for the inhibition of ferrocene compounds of **M** and **P** series of the BChE enzyme (**M1** to **M13** and **P1** to **P13**) was measured using standard methods (DNBT and absorbance at 412 nm. The *in-vitro* IC_{50} (µM) of BChE was determined by plotting the absorbance against concentration, and calculate the IC_{50} (µM) which is plotted for convenient comparison.

***In-Silico* BChE interaction studies:** To explore and illustrate the structure activity relationship among the synthesized compounds, docking experiments and an *in-Silico* study was performed on all synthetic compounds as summarized in the manuscript. The pdb structure of butyrylcholinesterase bound to an inhibitor (BChE 4TPK) was downloaded from the protein data bank (Brus, B.; Košak, U.; Turk, S.; Pišlar, A.; Coquelle, N.; Kos, J.; Stojan, J.; Colletier, J.-P.; Gobec, S. Discovery, biological evaluation, and crystal structure of a novel nanomolar selective butyrylcholinesterase inhibitor. *Journal of medicinal chemistry* **2014**, *57*, 8167-8179.) and the X-ray structures of the ferrocene compounds (**M7** and **P9**) were generated from the cif files using the Mercury software package. The **M1-M13** and **P1-P10** structures were obtained by modifying **M7** or **P9** using ChemUltra 3D software (From Cambridge Soft Inc.) (Ultra, C. 6.0 and chem3d ultra. *Cambridge Soft Corporation, Cambridge, USA* **2001**.). The compounds were then docked using the online server of Patch Dock and MolDock (Thomsen, R.; Christensen, M.H. Moldock: A new technique for high-accuracy molecular docking. *Journal of Medicinal Chemistry* **2006**, *49*, 3315-3321). The parameters used were Clustering RMSD = 2.0 and Complex type = Enzyme-Inhibitor. Docking results were analyzed by using Molegro Molecular Viewer and/or MolDock and LigPlot+ (Laskowski, R.A.; Swindells, M.B. Ligplot+: Multiple ligand–protein interaction diagrams for drug discovery. ACS Publications: 2011) software as previously described (Gull, Y.; Rasool, N.; Noreen, M.; Altaf, A.A.; Musharraf, S.G.; Zubair, M.; Nasim, F.-U.-H.; Yaqoob, A.; DeFeo, V.; Zia-Ul-Haq, M. Synthesis of n-(6-arylbenzo [d] thiazole-2-acetamide derivatives and their biological activities: An experimental and computational approach. *Molecules* **2016**, *21*, 266, pp 1-17). The parameters selected are defined as: PatchDock score that was calculated by Geometric shape complementarity considerations in the online PatchDock server (Duhovny, D.; Nussinov, R.; Wolfson, H.J. In *Efficient unbound docking of rigid molecules*, International workshop on algorithms in bioinformatics, 2002; Springer: pp 185-200; Schneidman-Duhovny, D.; Inbar, Y.; Nussinov, R.; Wolfson, H.J. Patchdock and symmdock: Servers for rigid and symmetric docking. *Nucleic acids research* **2005**, *33*, W363-W367); MolDock score and H-bond energies were calculated in Molegro Molecular Viewer. In this way MolDock score and H-bond energies are simulated by considering the following mathematical expressions (I, II and III) according to Thomsen and Christensen (Thomsen, R.; Christensen, M.H. Moldock: A new technique for high-accuracy molecular docking. *Journal of Medicinal Chemistry* **2006**, *49*, 3315-3321).

$$E_{\text{score}} = E_{\text{inter}} + E_{\text{intra}} \quad \text{(I)}$$

In (1) E_{inter} is the ligand-protein interaction energy and E_{intra} is the internal energy of the ligand. The mathematical expressions for E_{inter} and E_{intra} are as described by Thomsen and Christensen shown in equation (II) and (III). Readers are referred elsewhere for more details (Thomsen, R.; Christensen, M.H. Moldock: A new technique for high-accuracy molecular docking. *Journal of Medicinal Chemistry* **2006**, *49*, 3315-3321):

$$E_{\text{inter}} = \sum_{i \in \text{ligand}} \sum_{j \in \text{protein}} \left[E_{\text{PLP}}(r_{ij}) + 332.0 \frac{q_i q_j}{4 r_{ij}^{\,2}} \right] \quad \text{(II)}$$

$$E_{\text{intra}} = \sum_{i \in \text{ligand}} \sum_{j \in \text{ligand}} E_{\text{PLP}}(r_{ij}) + \sum_{\text{flexible bonds}} A[1 - \cos(m \cdot \theta - \theta_0)] + E_{\text{clash}} \quad \text{(III)}$$

Most of the **P** series compounds do not engage in H-bonding with the enzyme active site, and hence liberate zero H-bond energy whereas the M-series compounds do have conformations that support H-bonding to the enzyme. We used the LIGPLOT diagrams (Figure 5) to investigate potential interactions of both series of compounds and these images are shown in the supplemental material of all the compounds. Docking **M12** and **P12** into the X-ray of BChE are described here as a representative result using the LIGPLOT images. In the Figure 5 and the case of **M12**, the amino acids in the binding site providing the hydrophobic environment Asn68, Gln71, Leu88, Leu273, Ala277, Asn479, Asn481 and Pro491 (presented by red colored capping half circles with vertical lines) around **M12**. The amino acids Ile69 and Ser489 form NH---O type H-bonds (presented by green colored dash lines) with distances 3.03Å and 3.23Å respectively. Similar results are shown for **M2** and **M10**. **M2** and **M12** support two H-bonds, whereas **M10** is only involved in one. Although based on the computational results **M12** may be considered as a slightly more potent inhibitor than **M2** and **M10** in line with the hydrofobic forces being important for this class of compounds, this conclusion is not supported by the EC_{50} values. We point here to the fact that these studies are investigating a protein-inhibitor system in which the inhibitors have a structure where there are so many more degrees of freedom because the compounds are so flexible. We have carried out data mining studies reported previously (McLauchlan,C.C.; Peters,B.J.;

Willsky,G.R.; Crans,D.C. Coord.Chem.Rev. 2015, 301−302,163-202), where the investigations were done exclusively using the X-ray structures. Considering the conformational flexibility of the compounds and the many structures that can form and associate with the protein as well as the assumptions involved by using the BChE 4TPK-inhibitor complex as the basis for this work. All the LIGPLOT images have been made from the compound associating with the active site using this structure as a starting point.

The docking scores of all the compounds are in the similar order and least helpful to establish correlation between theoretical and experimental results, however the calculated H-bond energies were found very useful in this regard. It was found during docking analysis that most of the **P** series compounds do not engage H-bonding with the enzyme active sites, and hence liberate zero H-bond energy. It seems from the docking analysis that H-bonding impact the activity of the **M**-series compounds but has no effect on the **P**-series. It can be seen in the LIGPLOT diagrams of all the compounds (Figure 5 from the manuscript was extended to include all compounds); M-series analogues make stronger interactions with BChE structure.

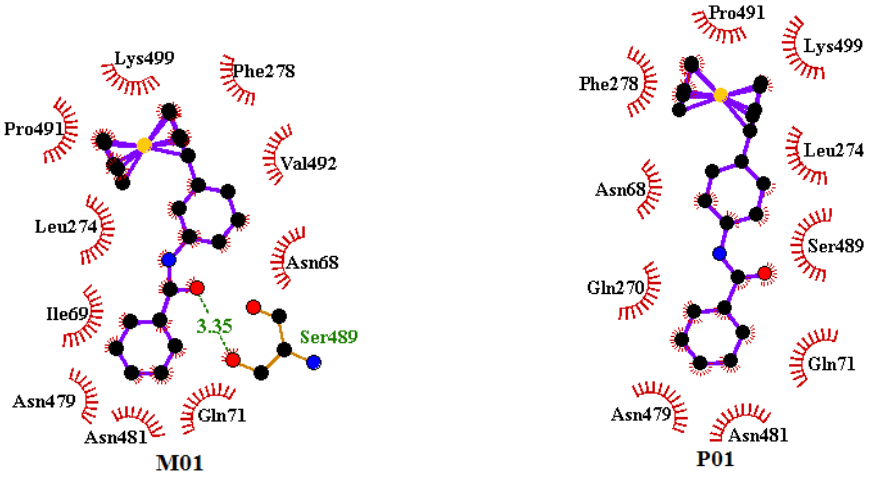


Figure 5: LIGPLOT images for the compounds **M1** to **M13** and **P1** to **P13** in the active site of BChE enzyme.

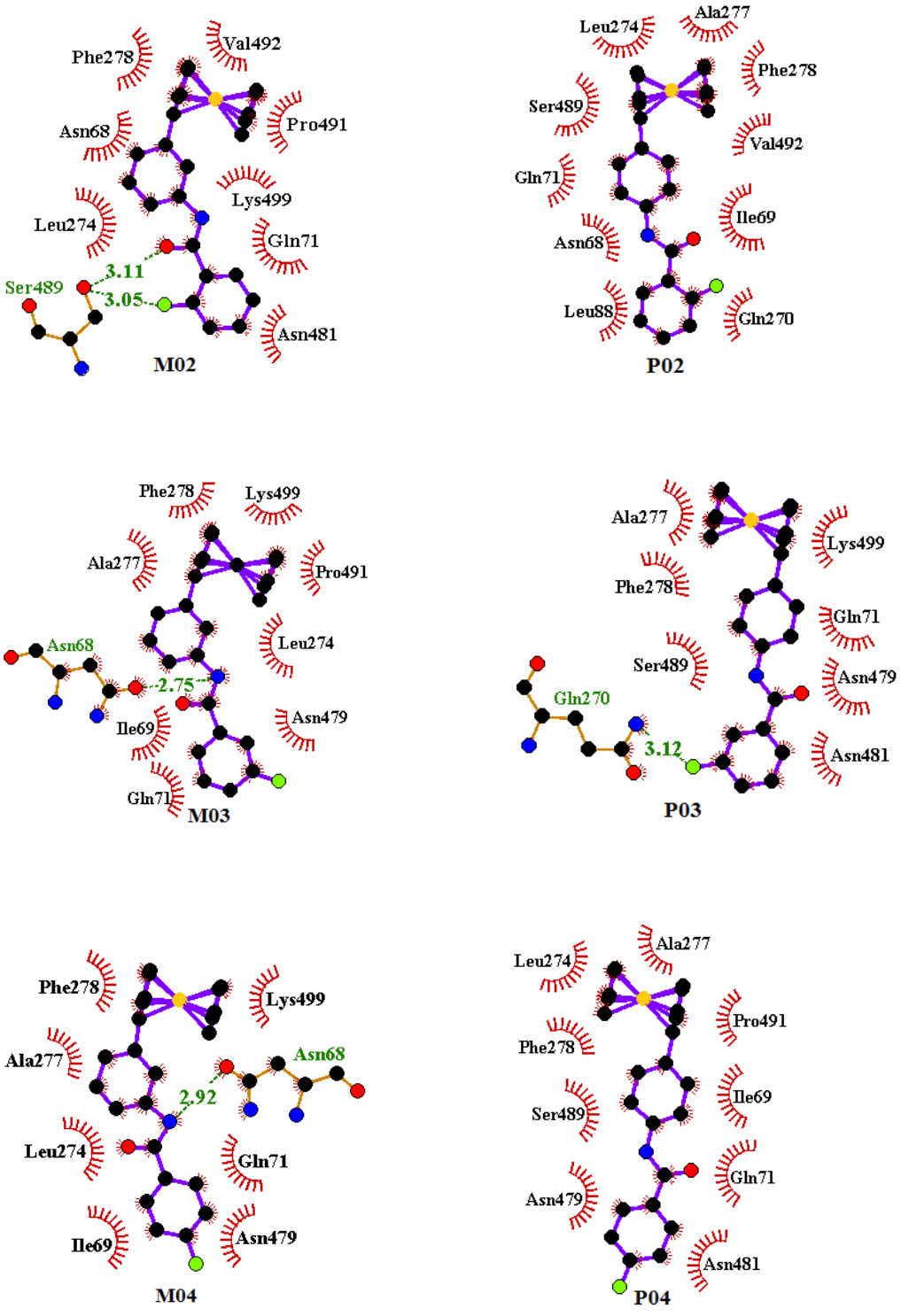


Figure 5: LIGPLOT images for the compounds **M1** to **M13** and **P1** to **P13** in the active site of BChE enzyme.

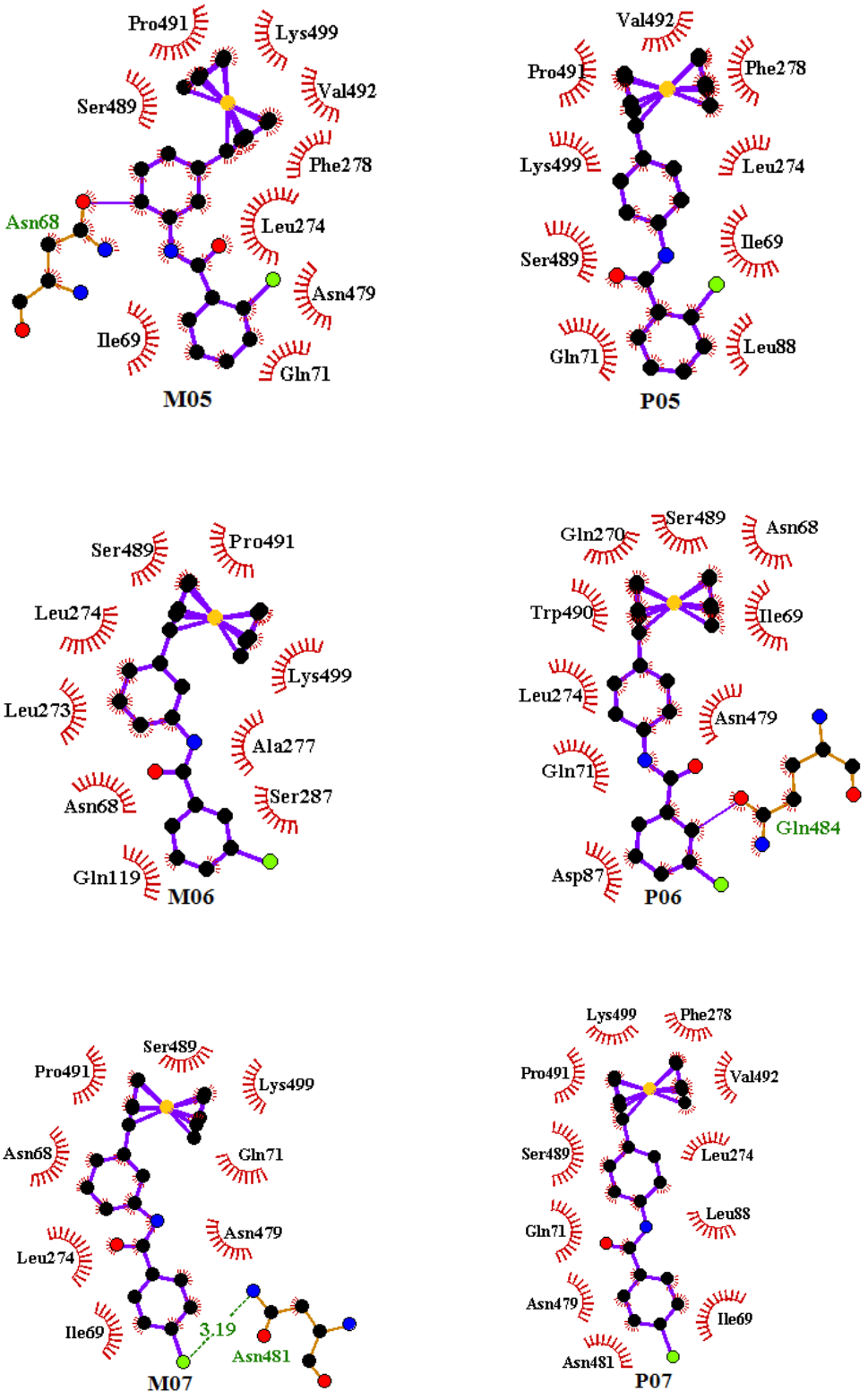


Figure 5: LIGPLOT images for the compounds **M1** to **M13** and **P1** to **P13** in the active site of BChE enzyme.

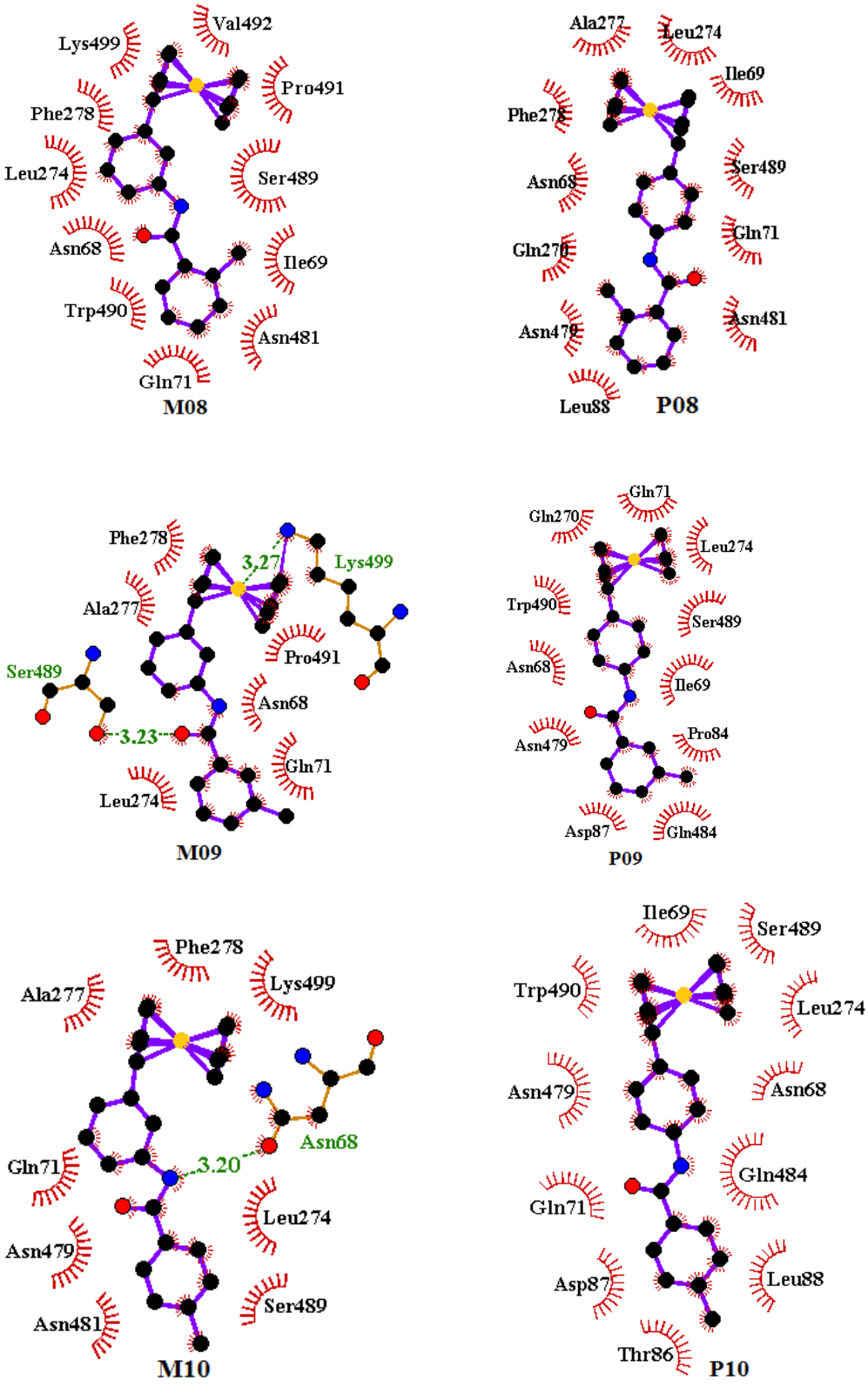


Figure 5: LIGPLOT images for the compounds **M1** to **M13** and **P1** to **P13** in the active site of BChE enzyme.

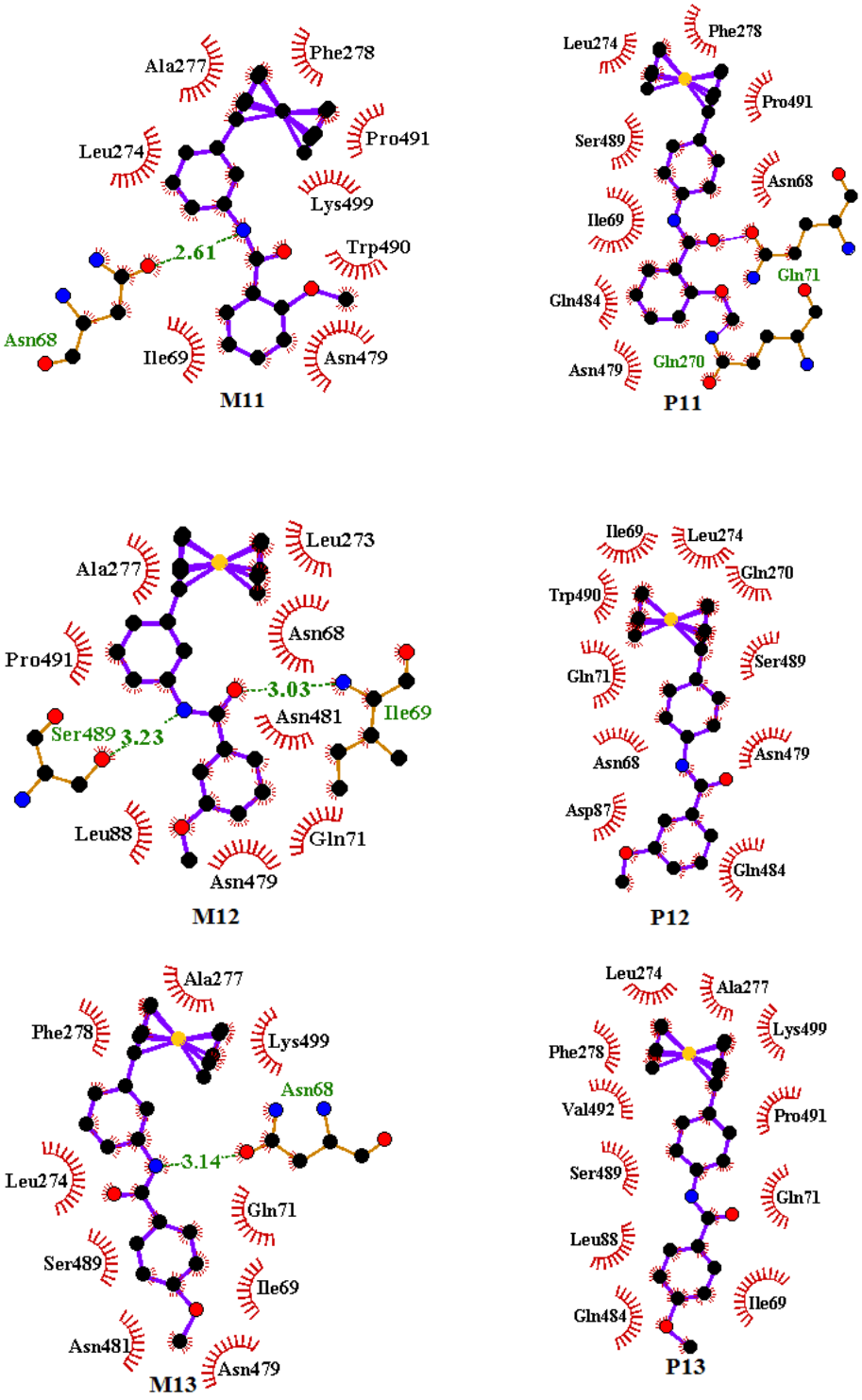
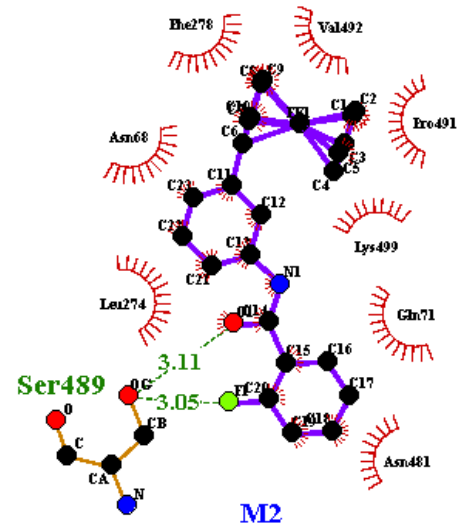


Figure 5: LIGPLOT images for the compounds **M1** to **M13** and **P1** to **P13** in the active site of BChE enzyme.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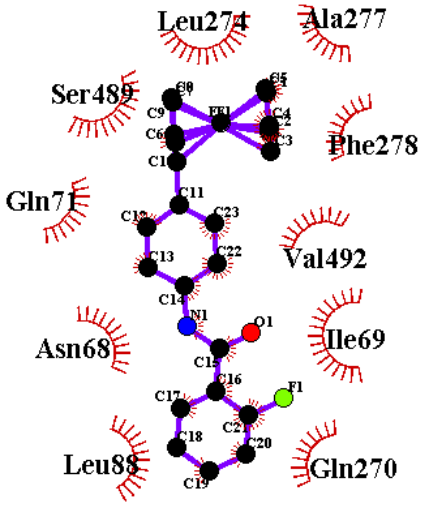
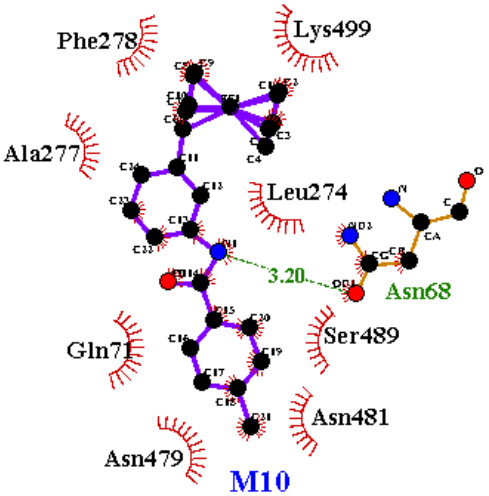
Asn68
Pro491
Lys499
Leu274
Gln71
3.11
Ser489
3.05
Asn481
M2


Leu274
Ala277
Ser489
Phe278
Gln71
Val492
Asn68
Ile69
Leu88
Gln270
P2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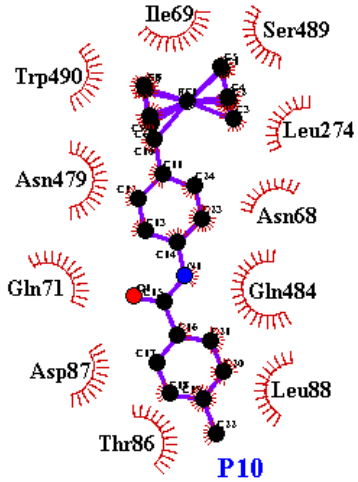
Lys499
Ala277
Leu274
3.20
Asn68
Ser489
Gln71
Asn481
Asn479
M10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Ser489
Trp490
Leu274
Asn479
Asn68
Gln71
Gln484
Asp87
Leu88
Thr86
P10

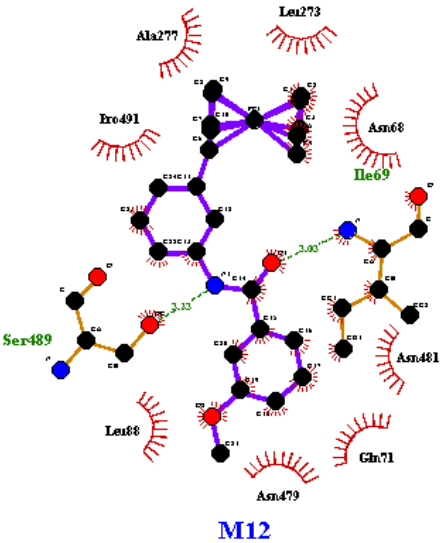
Leu273
Ala277
Pro491
Asn68
Ile69
Ser489
Asn481
Leu88
Gln71
Asn479
M12

Figure 6: LIGPLOT images for the compounds **M2, P2, M10, P10 and M12** in the active site of BChE enzyme in the presence of water solvent (there is no significant energy gain in the Plots in the absence and presence of water).

Docking of **M12** has been discussed here for the modeling analysis for the understanding of all the LIGPLOT images. In the figure 06 (**M12**), amino acids Asn68, Gln71, Leu88, Leu273, Ala277, Asn479, Asn481 and Pro491 makes hydrophobic interactions (presented by red colored capping half circles with vertical lines) with **M12**, hydrophobic interactions favor the protein binding with ligands having metal atoms. The amino acids Ile69 and Ser489 form NH---O type H-bonds (presented by green colored dash lines) with distances 3.03Å and 3.23Å respectively. All the LIGPLOT images are on the same pattern. There are some other kind of interaction in case of other molecules like, π --- π or π --- H interactions are represented by thin purple line as in figure 06 (**M5**) between amino acid Asn68 and **M5** molecule. The interaction between BChE and selected compounds (M2, P2, M10, P10 and M12) were also evaluated *in-silico* in the presence of water as solvent and results reported in Table 3 and ligplots (figure 5 and 6). These studies were done to explore the possibility that the presence of water would increase the role of the H-bond formation in this system. Thus, the compounds were investigated in several different orientations beginning from very different structures and allowing the system to optimize to the global minima. Examining the data demonstrate that there is no change in the interactions before (Figure 5) and after the presence of water (Figure 6). Only small values of water ligand interaction energies were found (as reported in table 3) supporting the interpretation that the effort of the H-bond is limited, and does not overcome the other stabilizing factors in the active site in these complexes.

The docking supported the experimental results to explain the greater inhibition of M – series over the P-series. But variations within the series are more difficult to correlate. Although Figure 7 below displays a linear correlation (R^2 = 0.923) between the IC_{50} values and H-bond energies such correlation has limited meaning when the differences in the inhibition are so small. Although such correlations have been reported in literature (Gull, Y.; Rasool, N.; Noreen, M.; Altaf, A.A.; Musharraf, S.G.; Zubair, M.; Nasim, F.-U.-H.; Yaqoob, A.; DeFeo, V.; Zia-Ul-Haq, M. Synthesis of n-(6-arylbenzo [d] thiazole-2-acetamide derivatives and their biological activities: An experimental and computational approach. *Molecules* **2016**, *21*, 266) such results must be viewed with causion in systems particularly like the one presented in this work where the biological responses are so small. We conclude that regardless of a attractive linear correlation

such as this shown below, if the effect observed with in the experimental data as that provided in this manuscript are minor, then computational methods will only provide limited insights to the analysis of the system.

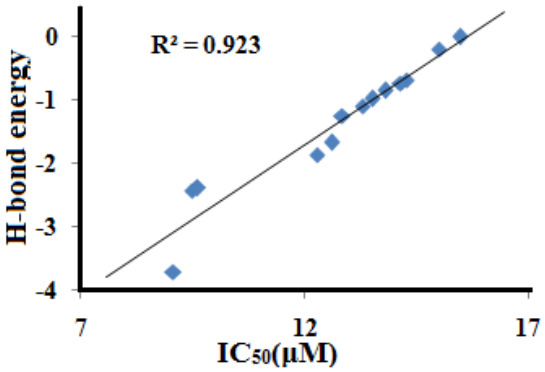

Figure 7: Correlation between mol dock predicted (by Molegro Molecular Viewer) H-bond energy and the *in-vitro* IC_{50} inhibition of the BChE enzyme by compounds **M1** to **M13**